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THE RESISTANCE SPECTRUM OF A DIELDIIN-RESSTRANT STRAIN OF THE BLOWFLY (*LUCILIA CUPRINA* WIED.).

BY

J. R. BUSVINE & G. J. SHANAHAN*)

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Strains of *Lucilia cuprina* showing resistance to dieldrin and aldrin appeared in Australia after 3 years successful use of these insecticides. In the laboratory these showed a "resistance spectrum" similar to that of *Anopheles gambiae, Cimex lectularius, Pediculus humanus* and *Musca domestica.*

For some three years prior to 1958, dieldrin and aldrin were successfully used in Australia to protect sheep from attacks of the blowfly *Lucilia cuprina* Wied. Towards the end of 1957, however, failures were occasionally reported in New South Wales. The preliminary studies of SHANAHAN (1958) conclusively proved that these failures were due to incipient insecticide resistance.

This setback naturally stimulated *ad hoc* research for alternative insecticides. At the same time, two kinds of basic study of this case of dieldrin resistance were initiated: toxicological investigations in Britain and genetical research in Australia. This paper concerns the former.

MATERIAL AND METHODS

Resistant and normal blowfly pupae were sent to Britain in 1958. Some preliminary experiments were done with the aim of delineating the resistance spectrum, by measuring relative resistance to a group of compounds toxicologically allied to dieldrin, such as aldrin, endrin, isodrin, chlordane and *gamma* BHC. The various compounds were dissolved in mineral oil (Shell Risella 17) and diluted to series of concentrations. Small drops (0.35 mm^3) were applied to the dorsal thoraxes of individual blowflies, as described by BUSVINE (1951) for houseflies.

This technique was found to work very well in producing straight, steep dosage/mortality curves for the normal colony. As regards the dieldrin-resistant colony, the tests showed that cross resistance extended to the other chlorinated cyclodiene compounds and to BHC. However, the dosage/mortality curves were very flat and irregular, as exemplified by the following results for dieldrin.

Dieldrin (%)	.0025	.005	.01	.025	.1	.2	.4	1.0	4.0
Kill (%)	10	28	40	52	40	60	70	76	86

It was concluded that the resistant colony was very heterogeneous and therefore quite unsuitable for estimating relative resistance levels.

---

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TABLE I

Results of tests with the susceptible colony, based on the following average number of flies per concentration: males 40; females 35

% concentration	Percentage mortality																				
	dieldrin			aldrin			endrin			isodrin			β chlordane			γ BHC			DDT		
	♂	♀	av.	♂	♀	av.	♂	♀	av.	♂	♀	av.	♂	♀	av.	♂	♀	av.	♂	♀	av.
.50																			97	98	97
.30																			78	75	76
.25																			87	48	68
.20																			50	27	38
.10													100	75	87	100	100	100	6	0	3
.05													71	37	54						
.04																84	70	77			
.03				96	93	94	100	100	100	100	100	100	19	5	12	78	41	54			
.025	100	100	100																		
.02	96	100	98	86	16	51	94	75	84	97	55	76				16	0	8			
.01	95	59	77	33	3	18	68	24	46	44	17	30									
.005	50	21	35																		
.0025	11	0	8																		
MLC	0.006			0.018			0.011			0.013			0.050			0.030			0.22		

Accordingly, it was decided to obtain, by laboratory selection, the homogeneous resistant colony necessary for both the toxicological and genetic work. This was done in Australia and the way in which it was accomplished is described by SHANAHAN (1960a). When it seemed that a pure resistant strain had been obtained, a colony was sent to Britain by air, in 1959. Since then, a sub-colony has been maintained, together with a susceptible one, at the London School of Hygiene and Tropical Medicine. The blowflies are kept at 27° C (80° F) in 12 inch cages, supplied with sugar and cotton wool pads soaked in milk and water (50 : 50). Eggs are laid on raw liver which is then put into jam jars and covered with sawdust. Pupae are sieved from the sawdust about 12 days later.

RESULTS

The results with the selected colony were now satisfactory, as shown by Tables I & II. Steep concentration/mortality curves (on logarithmic-probability paper) were obtained, allowing graphical estimation of the median lethal concentrations for most compounds. In a few cases the resistance was so high that 50% kill was not attained and the LC_{50} values are only very rough estimates obtained by extrapolation.

By comparison with LC_{50} figures for the susceptible colony, the following resistance levels were calculated:

dieldrin	× 670	endrin	× 36
aldrin	× (? 1400)	isodrin	× 54
β-chlordane	× (? 400)	γ -BHC	× 7.5

Resistance did not extend to DDT, towards which the dieldrin resistant colony

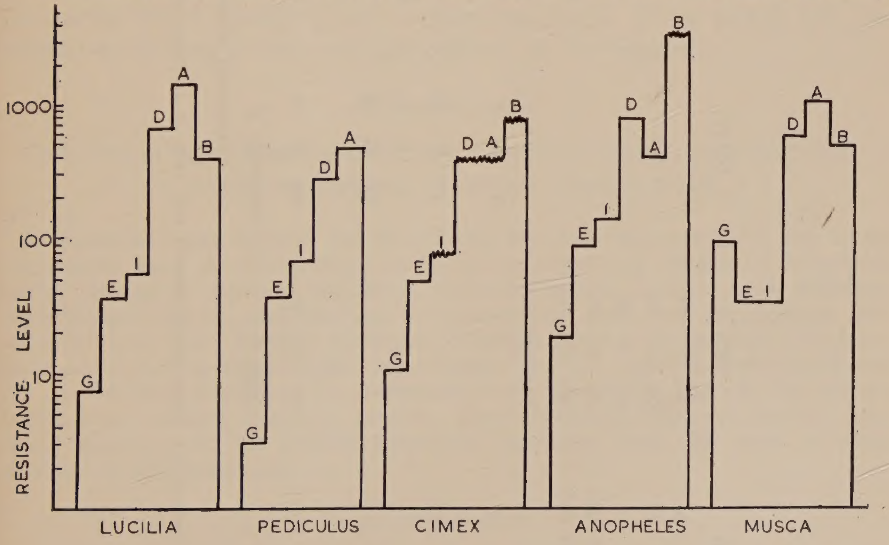

Figure 1. Resistance spectrum of *Lucilia cuprina* compared with those of other insects. *Pediculus humanus* (after BUSVINE & LIEN, 1960); *Cimex lectularius* (after BUSVINE, 1958); *Anopheles gambiae* (after DAVIDSON, 1958); *Musca domestica* (after BUSVINE, 1953). Insecticides: — G, *gamma* BHC; E, endrin; I, isodrin; D, dieldrin; A, aldrin; B. *beta*-chlordane.

TABLE II

Results of tests with the resistant colony, based on the following average numbers of flies per concentration: males 41; females 33

% concentration	Percentage Mortality																				
	dieldrin			aldrin			endrin			isodrin			β chlordane			γ BHC			DDT		
	♂	♀	av.	♂	♀	av.	♂	♀	av.	♂	♀	av.	♂	♀	av.	♂	♀	av.	♂	♀	av.
16				35	5	20															
10													45	21	33						
.8				6	0	3															
.5													26	6	15						
.4	76	26	52																		
.2	10	3	13							100	92	96	8	0	4						
.1	4	6	5				100	94	97	78	70	74				100	100	100			
.5							83	67	75	36	7	24				100	100	100	100	100	100
.3																93	88	90	100	100	100
.25							14	8	11							80	67	73			
.2																62	15	38	77	53	65
.1																13	0	6	4	0	2
MLC		4 0			(? 25)			0.4			0.7			(? 20)			0.22			0.17	

was rather more susceptible than normal (LC_{50}s 0.17% and 0.22%, respectively). Preliminary tests also showed no signs of resistance to parathion or malathion.

The data for the chlorinated cyclodiene compounds and for *gamma* BHC can be plotted graphically as a resistance spectrum. Figure 1 shows this in comparison with similar spectra for resistant strains of other insects. It is evident that there is considerable similarity, the inference being that the same primary defence mechanism responsible for resistance to this group of compounds can occur in widely different species of insect.

CONCLUSIONS

It is not to be expected that a relatively simple investigation such as this will provide sufficient insight into a case of resistance to suggest a way of overcoming it. What has been established is that dieldrin resistance in *Lucilia cuprina* is of the same type as that found in *Anopheles gambiae*, *Cimex lectularius*, *Pediculus humanus* and *Musca domestica*. It is also of interest that recent work of SHANAHAN (1960b) indicates that inheritance of the resistance in *L. cuprina* is similar to that in *A. gambiae*. These facts allow certain predictions about the potential growth and spread of the trouble, by analogy with happenings in other insects. Furthermore, the blowfly resistance is now seen not as an isolated phenomenon, but as one more example of a large problem which is the subject of considerable research. Any advance in this field, then, may be applicable to the problem of resistance in the sheep blowfly.

On the debit side, it must be admitted that the identification of this resistance as a typical dieldrin-BHC form, stigmatises it as an intractable nuisance, which renders ineffective a whole group of potent insecticides. At the present time, no satisfactory solution of this particular problem can be visualised.

ZUSAMMENFASSUNG

*DAS RESISTENZSPEKTRUM EINES DIELDRINRESISTENTEN STAMMES DER SCHAFSCHMEISSFLIEGE (*LUCILIA CUPRINA *WIED.)*

In Australien waren Dieldrin und Aldrin etwa drei Jahre lang erfolgreich zum Schutz von Schafen gegen die Schmeißfliege *Lucilia cuprina* angewendet worden, als Fehlschläge infolge einsetzender Resistenz aufzutreten begannen. Stämme normaler und resistenter Schmeißfliegen wurden gezüchtet und im Laboratorium untersucht. Zweigkolonien einschließlich eines durch Selektion bereinigten resistenten Stammes wurden nach London gesandt und Kreuz-Resistenzmessungen vorgenommen. Das sich ergebende Resistenzspektrum für die BHC/Dieldrin-Gruppe der Insektizide erwies sich ähnlich dem von vier anderen Insektenarten, nämlich *Anopheles gambiae*, *Cimex lectularius*, *Pediculus humanus* und *Musca domestica*, die den gleichen Resistenztyp entwickelt haben. Das weist auf einen ähnlichen Abwehrmechanismus hin.

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BUSVINE, J. R. & LIEN, J. (1960), Methods for measuring susceptibility to Insecticides in Bed Bugs, Cone-nose Bugs, Fleas and Lice. *Bull. Wld Hlth Org.* (in press).

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—— (1960b), Genetics of Resistance to Dieldrin in *Lucilia cuprina* Wied. *Nature, Lond.* **186** : 181.

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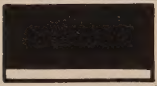
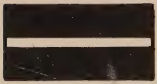
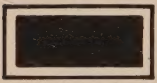
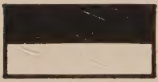
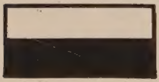
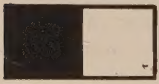
ERRATUM

In the paper:

BARRASS, R. (1960), The settling of female *Haematopota insidiatrix* Austen (Diptera, Tabanidae) on cloth screens. *Ent. exp. & appl.*, 3, 257—266, Table III, p. 262 should be read as follows:

Arrangement of Black and White		Ratio of flies on B.W.: on control		X^2	P	Date
		Observed ratio	Expected ratio			
	1)	35 : 37	33 : 39	0.224	<0.75	8.12.59
	2)	22 : 28	23 : 27	0.080	<0.9	12. 1.60
	1)	21 : 27	22 : 26	0.084	<0.9	16.12.59
	2)	23 : 41	29 : 35	2.270	<0.25	12. 1.60
	1)	26 : 25	17 : 34	7.147	<0.01	10.12.59
	1)	48 : 80	43 : 85	0.833	<0.50	9. 1.60
	1)	22 : 33	18 : 37	1.321	<0.25	13.12.59
	2)	9 : 41	17 : 33	5.705	<0.025	9. 1.60
	1)	24 : 31	18 : 37	2.793	<0.1	14.12.59
	2)	57 : 66	41 : 82	9.366	<0.005	9. 1.60
	1)	16 : 34	17 : 33	0.088	<0.9	15.12.59
	2)	26 : 40	22 : 44	1.091	<0.5	9. 1.60

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LIPOID CONTENT AND RESISTANCE IN THE HOUSEFLY, *MUSCA DOMESTICA* L.

BY

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The lipoid content of several resistant and normal strains of houseflies was determined. A Swiss extremely DDT-resistant strain had a much higher fat content than a normal Swiss reference strain and several Italian strains. Among the Italian strains, a chlordane-resistant strain had a significantly higher fat content than a diazinon-resistant and a normal strain. This difference was no longer significant however, when the normal Swiss strain was included in the calculations. Iodine numbers, specific gravity and physical consistence showed no consistent difference between the fats of resistant and normal strains, except that the syphoned-off oils from fats of resistant flies seemed to be less viscous than those of normal flies of the same provenience.

In a further series of experiments, the lipids were extracted from wheat bran, to which were added instead fats extracted from normal and from DDT-resistant housefly pupae; normal larvae were reared in a medium containing the fat from resistant pupae and vice versa. Neither this procedure nor that of offering cholesterol as sole larval lipid source, had any influence on resistance level in the adult state.

The tolerance level towards insecticides varies in the developmental stages of the insect, at different periods of its adult life (e.g. at hibernation) and according to its sex. It has often been tried to ascribe these changes in response to insecticides to characteristic differences in fat content. Attempts to correlate insecticide resistance with the internal lipoid content of insects are also rather numerous. Studies supporting such views have been published on *Agelastica alni* L. and *Pseudophonus pubescens* Müll. (BERIM & EDELMAN 1949, cited by LOFGREN & CUTCOMP 1956), *Melolontha melolontha* L. (LANDA, NOVÁK & SKUHRAVÝ 1952 and SKUHRAVÝ, NOVÁK, HRDÝ & HURKA 1953, cited by BROWN 1959), *Leptinotarsa decemlineata* Say (LANGENBUCH 1954, 1955), *Oryzaephilus surinamensis* L. (MUNSON, PADILLA & WEISSMANN 1954), *Periplaneta americana* L. (MUNSON 1953; MUNSON & GOTTLIEB 1953; MUNSON, PADILLA & WEISSMANN 1954), *Anopheles quadrimaculatus* Say (unpublished results of JONES, cited by MUNSON, PADILLA & WEISSMANN 1954), anophelines of the *maculipennis* group, such as *Anopheles typicus* Hackett & Missiroli, *Anopheles melanoon subalpinus* Hackett & Lewis, *Anopheles messea* Falleroni (DE ZULUETA, JOLIVET, THYMAKIS & CAPRARI 1957), *Anopheles atroparvus* Thiel (NERI, ASCHER & MOSNA 1958, 1959; GARMS, WEYER & REHM 1959) and about *Drosophila melanogaster* Meig. (MUNSON, PADILLA & WEISSMANN 1954; WIESMANN & REIFF 1956).

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On the other hand, results not confirming a correlation between resistance, tolerance or hardiness and lipoid level have been communicated for *Anthonomus grandis* Boh. (Reiser, Chadbourne, Kuiken, Rainwater & Ivy 1953 — note however a different interpretation of these data by Munson, Padilla & Weissmann 1954), *Periplaneta americana* L. (Lofgren & Cutcomp 1956), *Aedes aegypti* L. (Doby, Deblock & Gaeremynck 1956) and *Anopheles gambiae* Giles (Bradbury, Campbell & O'Carrol 1958).

In the housefly, *Musca domestica* L., the pioneer work of Mer & Furmanska (1953), who had noted a strong increase in DDT-hardiness of adults fed with milk containing butterfat or peanut oil, was followed by the extensive investigations of Wiesmann & Reiff. After having demonstrated that enzymatic dehydrochlorination was not an important factor in protecting the flies from the toxic action of DDT, at least in their DDT-resistant laboratory strains of houseflies, they discovered that two DDT-resistant strains contained (calculated on fresh weight) 13.0% and 14.9% respectively of total lipoids, while a normal reference strain had only 11.6%. Strains selected with other insecticides also showed enhanced lipoid level. The lipoids of resistant houseflies were less viscous, contained less precipitations of solid waxes and seemed to be of a less saturated character than those of normal flies. These differences in quantity and quality (followed up also in the tarsi and the thoraxic ganglia of strains resistant to different insecticides) were envisaged to allow storage of DDT in the lipoids of resistant houseflies and thus prevent its toxic action (Wiesmann 1955, 1957; Wiesmann & Reiff 1956; Reiff 1955, 1956, 1958 — last detailed reviews in Wiesmann 1958; Reiff & Beye 1960). These interesting studies have brought about, that Wiesmann, the discoverer of DDT-resistance in the housefly in 1947, is considered today as the main exponent (cf. Bradbury, Campbell & O'Carrol 1958) of the "fat barrier" theory of resistance, i.e. a protection mechanism of resistant insects from the effects of insecticides by storage in the fat body. Wiesmann's findings on the different quantity and quality of the fats of resistant houseflies have not been confirmed in gamma-BHC-resistant houseflies by Bradbury, Campbell & O'Carrol (1958). Also Micks & Singh (1958) found no difference in fat content (Soxhlet extraction) between strains of normal and resistant houseflies. The differences noted by these authors between the infrared absorption spectra of the acetone extracts of various strains, though attributed to content of specific lipids, were not linked with resistance. Finally Bridges & Cox (1959) stated that no significant difference was found in the total fat content of their housefly strains R (dieldrin-resistant), RND (R-strain in which dieldrin pressure was relaxed) and S (normal).

It was thought of significant interest to follow up the problem of lipoid content and resistance in our laboratory-reared strains of houseflies. For convenience sake, the determinations were carried out on housefly pupae.

MATERIAL AND METHODS

Strains of houseflies and rearing procedure

The following strains of *M. domestica* were used:

Swiss strains

Sv — normal reference strain (from Lungern), obtained from Dr. G. Saccà.
K_1 — extremely DDT-resistant strain (Ascher & Kocher 1954; Ascher 1957), obtained at Rome in 1957 from the J. R. Geigy Co., Basle* and kept since then under continuous selection pressure (DDT) in the larval and adult stages.

Italian strains

S-Rome — susceptible (cf. Ascher 1958).
R-Sard. — highly chlordane-resistant (Ascher 1958).
PRlp — highly diazinon-resistant, developed by Dr. G. Saccà (See Saccà 1959, 1959a).

Rearing of strains

The cultures were kept in a constant temperature room (27° C). The larvae were reared in great enamelled metal basins (upper diameter 50 cm, lower diameter 25 cm, height 13 cm) closed with a cloth cover. Each basin contained 3.125 kg medium, made up as follows:

1 kg wheat bran
125 g milk powder
2 liters of water

There was added to each basin 0.6 ml of housefly eggs (this amounts to about 4200 eggs — see Moreland & McLeod 1956). One, two and three days after addition of the eggs, the medium was thoroughly mixed once a day. One day before appearance of the first pupae (pupation commenced on the fifth day in K_1 and on the fourth day in all the other strains), the surface of the medium was somewhat pressed down, covered with dry sand for pupation and the cloth cover was removed. One day before the adults started to hatch (hatch commenced on the 9th day in K_1 and on the 8th day in all the other strains), the sand was sieved, the pupae thus collected were washed thoroughly to remove detritus sticking to them and were dried on filter paper sheets. The pupae were weighed and their approximate volume was determined in a 250 ml graduated cylinder. Under our breeding conditions there were obtained nearly always uniform pupae, which had the same size in whatsoever strain reared; 400 pupae reared under standard conditions occupied a volume of 20 ml.

Determination of fat content

The complete yield of pupae from one basin was always used for one determination. Only batches of pupae having the standard size, i.e. a volume, calculated for 100 g, ranging from 180—195 ml (see Table I), were employed. They were extracted in the Soxhlet apparatus, following in the initial steps the procedure of Anselmi & Cesari (1950): The pupae were ground in a glass mortar with twice their weight of anhydrous Na_2SO_4, the mixture was left for one hour in the covered mortar and was then transferred quantitatively into a paper package for Soxhlet extraction. The pupae were first extracted with ether (1) for 10 hours, after which the vessel with ether was exchanged for one with acetone. Re-extraction of the package with acetone (2) was then continued for another 7 hours. The greater part of the solvents was distilled off on the water bath, after which the ether and acetone extracts were dried first on the water bath at moderate temperatures and then at reduced pressure in vacuum exsiccators over $CaCl_2$ and solid paraffin wax until constant weight.

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*) This strain is identical with the R_k strain used by Wiesmann & Reiff (1956) in a great part of their determinations.

Determination of some chemical and physical properties of the extracted fats

These determinations were done only on the ether extracts, which had a liquid, oily consistence and contained nearly always some wax-like precipitates. Iodine number determinations were carried out with representative samples, obtained by vigorous shaking, of the whole ether extracts (mixture of oily phase and wax). The assays of physical properties (specific gravity and viscosity) were done with the syphoned-off liquid oil phase.

Iodine number. For the iodine number determinations the method of Wijs (Anon. 1942; Lepper 1950) was used. Each titration was repeated three times, of which the mean was calculated.

The specific gravity of the liquid phase was determined pyknometrically.

The viscosity of the liquid phase was determined with an Ostwald U-type viscosimeter at 25° and 27° C; each determination was repeated three times.

RESULTS

Determination of fat content

The results of the fat extractions are summarized in Table I.

TABLE I

Fat content of housefly pupae from different strains

Sample	Strain	Weight of batch, g	Volume of batch, ml	Estimated number fo pupae	Volume calculated for 100 g of batch	Lipoids extracted/fresh weight 1. Ether grams	%	2. Re-extraction-Acetone grams	%	Arithmetical mean of ether extracts, %
					Swiss strains					
A-1	Sv	106.9110	197	3940	184.3	6.1348	*5.738%*	1.1988	1.121%	
A-2	Sv	85.2616	164	3280	192.3	4.7929	*5.621%*	0.4630	0.525%	
A-3	Sv	58.4441	109	2180	186.5	3.2719	*5.598%*	0.3319	0.568%	
A-4	Sv	61.0398	116	2320	190.1	3.1186	*5.110%*	not done		5.544%
A-5	Sv	70.6329	135	2700	191.1	3.9365	*5.573%*	0.3726	0.528%	
A-6	Sv	95.8063	180	3600	187.9	5.3860	*5.622%*	0.3137	0.327%	
B-1	K1	78.9640	142.5	2850	180.5	5.5451	*7.021%*	not done		
B-2	K1	100.7865	190	3800	188.5	7.8506	*7.790%*	0.7560	0.750%	
B-3	K1	74.9490	137	2740	182.8	5.8725.	*7.835%*	0.6121	0.817%	7.316%
B-4	K1	78.1117	148	2960	189.5	5.4418	*6.967%*	0.4703	0.602%	
B-5	K1	97.8940	179	3580	182.8	7.0094	*7.160%*	0.3165	0.323%	
B-6	K1	62.1952	119	2380	191.3	4.4301	*7.123%*	0.3420	0.550%	
					Italian strains					
C-1	S-Rome	84.9157	161	3220	189.6	4.4792	*5.398%*	0.3925	0.462%	5.409%
C-2	S-Rome	100.5291	195	3900	194.0	5.4492	*5.420%*	0.3650	0.363%	
D-1	R-Sard.	89.7394	170	3400	189.4	5.2460	*5.846%*	0.2901	0.323%	5.854%
D-2	R.Sard.	88.7493	170	3400	191.6	5.2012	*5.861%*	0.4004	0.452%	
E-1	PRlp	64.3524	120	2400	186.5	3.4962	*5.433%*	0.4961	0.771%	
E-2	PRlp	66.2842	121	2420	182.5	3.7173	*5.608%*	0.3556	0.536%	5.459%
E-3	PRlp	70.8720	132	2640	186.3	3.7809	*5.335%*	0.3191	0.450%	

TABLE II

Statistical treatment of the figures of Table I

a. *Strains Sv and K_1*

	Sum of squares	degrees of freedom	mean square	"F"
Strains	9.43	1	9.43	94.3
error	1.01	10	0.10	—
Total	10.44	11	—	—

Tabled "F" for 1 and 10 degrees of freedom and 5% level of significance equals 4.96 (significant at < 0.001)

b. *Strains S-Rome, R-Sard. and PRlp*

	Sum of squares	degrees of freedom	mean square	"F"
Strains	0.25	2	0.12	12
error	0.04	4	0.01	—
Total	0.29	6	—	—

Tabled "F" for 2 and 4 degrees of freedom and 5% level of significance equals 6.94

c. *Strains Sv, S-Rome, R-Sard. and PRlp*

	Sum of squares	degrees of freedom	mean square	"F"
Strains	0.24	3	0.080	2.58
error	0.28	9	0.031	—
Total	0.52	12	—	—

Tabled "F" for 3 and 9 degrees of freedom and 5% level of significance equals 3.86

An "F" test was performed on the sets of results of the ether extraction from Swiss strains Sv and K_1 and separately for the Italian strains S-Rome, R-Sard. and PRlp. In the first test (Table II, a), K_1 was found to be highly significantly different in fat content from Sv. Pupae of strain K_1 contained about 32% more lipoids than the normal Swiss reference strain; this agrees well with the findings of WIESMANN & REIFF (1956) in adults of the R_k-strain (29%). In the second test (Table II, b) significant differences were found between the three Italian strains as well. Using SCHEFFÉ's (1953) method, it turned out, that R-Sard. was significantly different (at the 5% level) from S-Rome and PRlp. On comparing, however, the results of the normal Swiss reference strain Sv and the three Italian strains (Table II,c), the differences were no longer significant at the 5% level.

The only strain having a distinctly much higher lipoid content was thus the extremely DDT-resistant Swiss K_1-strain (Wiesmann's R_k strain). The chlordane-resistant strain R-Sard. had a significantly higher lipoid content only when compared with other Italian strains; this was no longer valid when also the normal Swiss reference strain Sv was included in the statistical treatment. The highly diazinon-resistant Italian strain PRlp had no enhanced fat content.

Iodine numbers

Some determinations of iodine numbers carried out with different samples (ether extracts) of fats from the Swiss strains Sv and K_1 yielded the following diazinon-resistant Italian strain PRlp had no enhanced fat content.

TABLE III

Iodine numbers of fats from strains Sv and K1

Sv	—	56.3, 51.1, 61.5, 64.3, 57.2, 60.7	K1	—	58.1, 60.5, 53.4, 60.6, 63.0, 51.6

Since thus no differences in iodine numbers were found between the fats extracted from the normal Swiss reference strain and from the extremely DDT-resistant strain K_1 (whose breeding colony was virtually during its whole life in contact with DDT), iodine number determinations were discontinued.

Specific gravity of the liquid phase

The following data were obtained at 25° C (Table IV):

TABLE IV

Specific gravity of syphoned-off oily fraction

Various samples of Sv	—	0.9074,	0.9078,	0.9141,	0.9153
Various samples of K1	—	0.9069,	0.9107,	0.9139,	0.9146
S-Rome	—	0.9076			
R-Sard.	—	0.9119			
PRlp	—	0.9113			

There were thus no consistent differences in specific gravity.

Viscosity of the liquid phase

The data (means of three determinations) are reported in Table V:

TABLE V

Viscosity of the syphoned-off oily fraction

	at 25° C	at 27° C
Sv	71.66 cp	66.19 cp
K1	53.31 cp	49.32 cp
S-Rome	63.99 cp	56.41 cp
R-Sard.	52.51 cp	48.60 cp
PRlp	56.39 cp	53.29 cp

It seems that the syphoned-off oils from fats of resistant strains have a somewhat lower viscosity than those of normal strains of the same geographical provenience. Indeed, already WIESMANN & REIFF (1956) had found that the acetone-extracted lipoids of resistant houseflies were less viscous than those of normal ones.

Consistence and aspect of ether extracted fats

WIESMANN & REIFF (1956) however also stated, that there was a heavier precipitate of solids in the fats of normal strains. We found that in our strains, resistant as well as normal, extracted fats varied in their consistence from batch to batch. They thus may or may not contain great quantities of precipitated wax, irrespective of resistance (Table VI).

TABLE VI

Consistence of ether-extracted lipoids

Sv	K_1	S-Rome	R-Sard.	PRlp
+ − + + + +	+ − − + − +	+ +	+ +	− + +

+ sample contained solid waxes
— sample was limpid

It was noted, however that the precipitate always seemed much less voluminous in K_1, when batches of fats, containing precipitated wax, from the two Swiss strains Sv and K_1 were compared. Therefore there was attempted an estimation of these waxes in a precipitate containing sample of K_1 and two such samples of Sv. The samples were shaken well, until they had a homogeneous appearance. Then the major part of them was poured into graduated centrifuge tubes of known weight and each sample was centrifuged for 15 minutes at 2500 rpm. All the determinations were carried out at 25° C (Table VII).

TABLE VII

Estimation of wax precipitated in samples of lipoids of K_1 and Sv

Strain	*K_1*	*Sv*	*Sv*
Sample	B-2	A-1	C-1
Volume of substance in the tubes	8.2 ml	5.3 ml	5.0 ml
Weight of substance in the tubes	7.3928 g	4.8845 g	4.4559 g
Appr. volume of wax ppt.	0.18 ml	0.3 ml	0.3 ml
Appr. volume of supernatant oil	8.02 ml	5.0 ml	4.7 ml
Specific gravity of supernatant oil	0.9069	0.9074	0.9078
Appr. weight of supernatant oil	7.2733 g	4.5370 g	4.2666 g
Appr. weight of wax ppt.	0.1195 g	0.3475 g	0.2893 g
Appr. percentage of wax ppt.	*1.6%*	*7.1%*	*6.5%*

Solubility of DDT in syphoned-off oils

Only some preliminary experiments were done on the solubility of DDT in the syphoned-off liquid phase of the ether-extracted fats of strains Sv and K_1. These experiments seemed to indicate that the solubility at 27° C was 9% in both cases. No determinations as to the respective speeds of solution were undertaken.

Influence of lipids in larval diet on the resistance of adult houseflies

It has been demonstrated that feeding or injecting of lipids reduced the susceptibility of adult normal houseflies to DDT (MER & FURMANSKA 1953; WIESMANN & REIFF 1956). The effect on adults of fortifying normal larval media by *addition* of lipids, such as cod liver oil, olive oil and also cholesterol, was also investigated. The resistance level of adults of a DDT-sensitive strain, grown from these larval breeding mixtures, was not enhanced (WIESMANN & REIFF 1956). Since these experiments could not, however, answer the question, whether *different* fats in the larval medium could bring about a change of resistance in the adults, the following experiment was devised: Ether-extracted fats from K_1 and Sv pupae were offered as exclusive lipid source to larvae in such a way that K_1 (resistant) fat was offered to Sv larvae and Sv (normal) fat to K_1 larvae. For this purpose there was prepared a lipid-free medium (LFM) by extracting wheat bran at least 20 times with chloroform (LEVINSON & BERGMANN 1957) in the Soxhlet apparatus. The following mixtures were made up in 400 ml beakers, stirred well and covered with filter paper:

1) 15 g LFM + 0.75 g fat extr. from K1 + 120 ml ether
2) 15 g LFM + 0.75 g fat extr. from Sv + 120 ml ether
3) 15 g LFM + 120 ml ether (Control)
3a) 15 g LFM + 120 ml ether (Control)

After complete evaporation of the ether, to each beaker 200 housefly eggs were added, which had been deposited on cotton wool moistened with water, the milk having been removed from the breeding cages the previous day. The eggs were suspended, according to LEVINSON & BERGMANN (1957) in 30 ml of 5% yeast autolysate (lipid-free, of the firm Dr. Piccioni, Brescia, Italy) in distilled water; this was mixed well with the LFM preparations. The formulations were inseminated with eggs as follows:

1) Medium containing fat from K1 — with 200 eggs of Sv
2) Medium containing fat from Sv — with 200 egss of K1
3) Lipid-free control — with 200 eggs of Sv
3a) Lipid-free control — with 200 eggs of K1

The beakers were covered with filter paper and incubated at 30° C. The following yield of pupae was obtained:

In 1) there were obtained 132 pupae of Sv;
in 2) there were obtained 168 pupae of K1;
in 3) and 3a) all larvae died in the first or second stage (due to lack of sterol, cf. SILVERMAN & LEVINSON 1954; LEVINSON & BERGMANN 1957).

On hatch, the adults were fed for 3—4 days on sugar and water and then the females were brought into 2 hours continuous tarsal contact with well crystallized (REUTER & ASCHER 1956) layers of 0.5 g/sq.m. DDT (for full details of method see ASCHER 1957). The results were as follows (Table VIII):

TABLE VIII

Replacement of lipids offered to larvae in the medium and adult resistance

(0.5 g/sq.m. DDT in Petri dishes; T = 27° C; each experiment with 4 × 10 female houseflies, 3—4 days old)

Medium:	1	2	normal wheat bran	
Fly strain:	Sv	K1	Sv	K1
Contact, minutes	Percentage of knock-down			
10	—	—	—	—
20	67	—	50	—
30	87	—	100	—
60	100	—	100	—
90	100	—	100	—
120	100	—	100	—
Σ% k.d.	454	0	450	0

Adult resistance levels thus remained unaltered.

Influence of cholesterol in larval diets on the resistance of adult houseflies

Cholesterol has been claimed in an earlier work of WIESMANN (1955) to have some function in housefly resistance to DDT. He noted that the cholesterol content of nervous tissues was 2—3 times higher in resistant than in susceptible flies. BERGMANN, LEVINSON & MECHOULAM (1958) found that addition of 5000 ppm cholesterol to wheat bran somewhat inhibited the toxicity of 0.5 and especially of 1.0 ppm protoveratrine in the diet to housefly larvae. It was therefore deemed of interest to exchange the wheat bran lipids by 50, 500 and 5000 ppm of cholesterol in the larval diets and to assay the response of the adults grown thereon to DDT. Again eggs of both a resistant (K_1) and a normal (Sv) Swiss strain were used. The following media were prepared in 400 ml beakers in double replication (chol. = cholesterol, Y = yeast):

1) 15 g LFM + 2.25 mg chol. + 120 ml ether
2) 15 g LFM + 22.5 mg chol. + 120 ml ether
3) 15 g LFM + 225 mg chol. + 120 ml ether
4) 15 g LFM + 120 ml ether (LFM Control)
5) 15 g normal wheat bran + 120 ml ether (wheat bran Control)

After the evaporation of the ether, there were added to each formulation 200 eggs of Sv and K_1 respectively; the eggs had been suspended again in 30 ml of 5% yeast autolysate. The following yield of pupae was obtained:

	Sv	*K*₁
1) LFM + 50 ppm chol. + Y	68 pupae	166 pupae
2) LFM + 500 ppm chol. + Y	156 pupae	155 pupae
3) LFM + 5000 ppm chol. + Y	110 pupae	160 pupae
4) LFM + Y (LFM Control)	0 pupae	0 pupae
5) normal wheat bran + Y (wheat bran Control)	90 pupae	160 pupae

The results of the knock-down experiments with the adults on DDT are summarized in Table IX. In experiment 1) of strain Sv only 3 × 10 adult females were used and in all the other experiments 4 × 10. The test procedure was the same as described in the former series.

TABLE IX

Cholesterol in larval media and adult resistance

Medium:	1	2	3	5	1	2	3	5
Fly strain:	Sv				K1			
Contact, minutes	Percentage of knock-down							
10	40	40	35	—	—	—	—	—
20	87	100	95	44	—	—	—	—
30	100	100	100	94	—	—	—	—
60	100	100	100	100	—	—	—	—
90	100	100	100	100	—	—	—	—
120	100	100	100	100	—	—	—	—
Σ% k. d.	527	540	530	438	0	0	0	0

Even massive doses of cholesterol offered as sole lipid source in the larval diet did not influence adult resistance levels.

DISCUSSION

The storage of toxicants in the lipoids of resistant insects, as suggested by WIESMANN & REIFF (1956), seemed to provide us with an explanation of the mechanism of resistance, which would be plausible especially in strains in which other mechanisms (e.g. of detoxication such as enzymatic dehydrochlorination of DDT) could not be demonstrated. The storage mechanism seemed the more acceptable inasmuch as the studies of the Swiss authors pointed out not only an enhanced lipoid content of the tarsi, the thoraxic ganglione complex, the flies' whole epicuticle and the tarsal epicuticle, but also established higher iodine numbers of the lipoids in resistant flies, which would result in better DDT dissolving capacity.

We have shown in earlier work (NERI, ASCHER & MOSNA 1958, 1959) that indeed in several laboratory developed DDT-resistant strains of *Anopheles atroparvus* Thiel there occurred such an enhanced fat content. But in the housefly, Wiesmann's work could not be confirmed by various authors. The present study arrives at the same negative conclusions: The only resistant strain, in which enhanced fat content could be clearly demonstrated, was the DDT-resistant Swiss strain, which had been used also in Wiesmann's investigations; in fact, our results on surplus total lipoid content of this strain agreed well with those of Wiesmann. It is clear, however, that neither the bulk, nor the physical properties of the fats of a diazinon and of a chlordane-resistant strain were such as to make a storage mechanism operative. In the housefly, Wiesmann's "fat barrier" theory, though perhaps serving a useful purpose in certain strains, is in no way of general validity.

The experiments in which it was attempted to change resistance levels of adults by offering different fats (derived from resistant and normal housefly pupae) or cholesterol as larval lipid source, yielded negative results. This was rather to be expected. Thus WIESMANN & REIFF (1956) are quite correct in stating on basis of their experiments:

"Wir stellen also fest, dass die durch Lipoide mitbedingte Resistenz bei den *R*-Fliegen erbgebunden ist und daher nicht durch erhöhte Fettnahrung der Larven verstärkt werden kann".

We can now add to this statement, that even by replacing the wheat bran lipids with fats of resistant and normal housefly pupae or with cholesterol in the breeding medium, no change was brought about in the resistance (or susceptibility) level of resistant and normal adults.

There are indications, that small changes in the resistance level of adults can be effectuated by certain manipulations in the adult stage itself. Feeding normal houseflies with milk fortified with butterfat or peanut oil (MER & FURMANSKA 1953) or injection of olive oil (WIESMANN & REIFF 1956) increases their hardiness towards DDT. On the other hand, injection of lipase into, or starvation of resistant adults reduces their resistance level. It has to be mentioned, however, that TAHORI (unpublished data) observed no change in response to DDT in DDT-resistant houseflies, which had been treated topically with dinitrotoluene or dinitrophenol 24 and 48 hours before the DDT application under the assumption that these compounds would accelerate the flies' metabolism.

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ZUSAMMENFASSUNG

LIPOIDGEHALT UND RESISTENZ DER HAUSFLIEGE, MUSCA DOMESTICA *L.*

Es wurde der Lipoidgehalt der Puppen resistenter und sensibler Hausfliegenstämme bestimmt. Ein hoch DDT-resistenter Schweizer Stamm hatte einen bedeutend höheren Fettgehalt als ein normaler Schweizer und verschiedene italienische Stämme. Unter den italienischen Stämmen, hatte ein Chlordan-resistenter Stamm einen signifikant höheren Fettgehalt als ein Diazinon-resistenter und ein normaler Stamm. Wenn jedoch bei der statistischen Auswertung dieser Resultate die Zahlen für den Schweizer normalen Vergleichstamm mit in Betracht gezogen wurden, waren die Unterschiede für den Chlordanstamm nicht mehr signifikant.

Die Feststellung Wiesmanns, dass resistente Hausfliegen einen höheren Fettgehalt aufweisen, konnte also in unseren Versuchen nur in einem von drei resistenten Stämmen bestätigt werden. Es ist daher anzunehmen, dass die Tatsache vorhandenen höheren Fettgehaltes nicht für alle resistenten Stämme zutrifft. Die Wiesmann'sche Lipoidtheorie der Insektizidresistenz, die übrigens auch von ihm selbst nur als Teilmechanismus des Resistenzgeschehens aufgefasst wird, ist vielleicht für gewisse Stämme anwendbar, so z.B. für den Schweizer Stamm K1, für den unsere Resultate mit den Ergebnissen Wiesmanns übereinstimmen. Die Theorie ist jedoch sicher nicht für jeglichen resistenten Hausfliegenstamm gültig.

Wenn chemische und physikalische Eigenschaften der Fette, z.B. Jodzahl, Dichte und Konsistenz verglichen wurden, konnten keine klaren Unterschiede zwischen dem Fett resistenter und normaler Stämme gefunden werden. Vielleicht könnte man nur von einem Unterschied in der Viskosität der abgeheberten flüssigen Ölphase resistenter und normaler Fliegen gleicher Provenienz sprechen.

Extrahierung der im Larvenfutter vorhandenen Fette und ihr Ersatz durch Fliegenpuppenfette, oder Verabreichung von Cholesterin als einzige larvale Fettquelle, hatte keinen Einfluss auf die Resistenz der Adulttiere.

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DIE WIRKUNG VON APHIDENSTICHEN AUF PFLANZLICHE ZELLEN

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Die Aphiden rufen durch den Einstich bei ihren Wirtspflanzen Reaktionen mannigfaltiger Art hervor. So sind uns Auswirkungen auf den Wasserhaushalt und den Stoffwechsel pflanzlicher Gewebe bekannt, die auf die Einwirkung pflanzensaugender Insekten zurückzuführen sind. Diese Reaktionen werden durch das Zusammenwirken der mechanischen und chemischen Komponente des Stiches ausgelöst.

Der Einstich der Aphiden in pflanzliche Gewebe stellt einen kombinierten Reiz mit einer mechanischen und einer chemischen Komponente dar. Der mechanische Reiz wird durch das Versenken der Mundwerkzeuge in das Gewebe verursacht, während der chemische Reiz durch die Speichelausschüttung ausgelöst wird. Wegen der Feinheit der Aphidenstechborsten wird im allgemeinen eine geringe mechanische Schädigung der Zelle angenommen, während die Hauptwirkung auf den Speichel zurückgeführt wird.

In den nachfolgenden Untersuchungen soll versucht werden, die Frage zu klären, welche Wirkung der Aphidenstich bzw. seine beiden Komponenten einzeln und kombiniert auf die pflanzliche Zelle besitzen. Diese Arbeit soll die Untersuchungen von KLOFT (1956) weiter fortführen und auf zytologisches Gebiet ausdehnen. Ihm gelang es u.a. unter Anwendung vielseitiger und moderner pflanzenphysiologischer Arbeitsmethoden, die Zusammensetzung von Aphidenspeichel, seine Verteilung im pflanzlichen Gewebe, seine Ausschüttung und Wirkung teilweise zu klären. Außerdem sei besonders auf die Arbeit von VAN HOOF (1958) hingewiesen, die im Zusammenhang mit Virus-Vektor-Problemen Einstich, Stichverlauf und Saugverhalten von Blattläusen untersucht.

EINE METHODE ZUR ZELLPHYSIOLOGISCHEN UNTERSUCHUNG DER WIRKUNG VON APHIDENSTICHEN

Für die nachfolgenden Versuche wurde eine Methode erarbeitet, die es entgegen früheren Untersuchungen erlaubt, die Wirkung von Aphidenstichen auf pflanzliche Zellen an lebendem Material zu beobachten.

Als Versuchstier wurde die Zwiebellaus, *Myzus ascalonicus* Donc., gewählt, die sich vorwiegend auf Pflanzen der Gattung *Allium* nährt. Aus dieser Pflanzengattung erschien uns die obere (konkave) Epidermis der Zwiebelschuppe von *Allium cepa* für die zytologischen Versuche am meisten geeignet (KLOFT & MAREK, 1955), da wir viele Grundtatsachen unserer Kenntnis von der lebenden Pflanzenzelle diesem so überaus günstigen Versuchsmaterial verdanken (STRUGGER, 1949).

Für die Untersuchungen benötigten wir isolierte Gewebeflächen, auf denen die Aphiden zum Anstich gebracht werden konnten. Deshalb wurde nach STRUGGER (1949) die Oberseite (konkave Seite) der Zwiebelschuppe in kleine Quadrate zerlegt, zur Erleichterung des Abhebens der Epidermis solange entlüftet, bis sie durchsichtig wurde (ungefähr 20 Sekunden), und zur Abschwächung des Wundreizes reichlich in fließendem Leitungswasser mehrere Stunden gewässert. Diese Wässerung ist unbedingt notwendig, da durch das Zerteilen der Zwiebelschuppe, selbst wenn dies mit einem noch so scharfen Rasiermesser geschieht, Verletzungen entstehen, die auch die Nachbarzellen beeinflussen können. Die Größe der Quadrate muß so gewählt werden, daß diese nach dem Abheben, ohne sich einzurollen, auf einen Objektträger gebracht werden können (STRUGGER, 1949). Nach der gründlichen Wässerung werden die Zwiebelschuppen auf ihrer Oberseite mit Filtrierpapier vom anhaftenden Wasser befreit und den Läusen bei Grünlicht zum Anstich vorgelegt. Die ganze Versuchskammer konnte mit Hilfe des Binokulars ständig kontrolliert werden. Hatten die Läuse angestochen, so wurde in die Versuchskammer ein gleichmäßiger, nicht zu starker und nicht auf die Läuse gerichteter CO_2-Strom eingeleitet, der die Läuse innerhalb von 3 Minuten betäubte. Tiere, die nicht genügend tief eingestochen hatten, zogen hierbei die Stechborsten aus dem Gewebe heraus, während die anderen in Saugstellung verblieben.

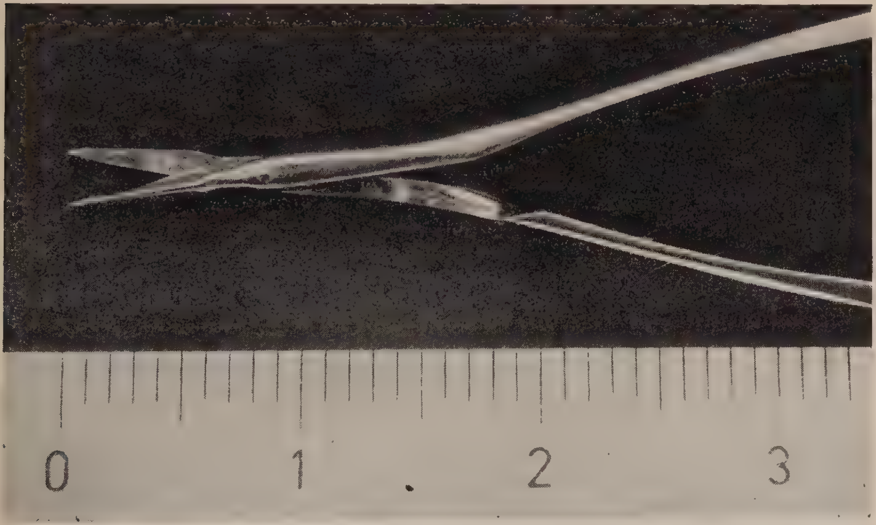

Abb. 1. Iridotomieschere nach Arruga

Die Epidermisschuppe wurde anschließend in eine Präparierschale gebracht und hier mit Hilfe einer Iridotomieschere nach Arruga (Abb. 1) der Rüssel abgeschnitten. Anschließend konnte die Epidermis vorsichtig abgehoben werden. Der Rüssel blieb in der Epidermis stecken und diente als Markierung der Einstichstelle (KLOFT & MAREK, 1955). Es wurde darauf geachtet, daß die Läuse in der Mitte der Quadrate eingestochen hatten. Auch wurden nur diejenigen Schuppen verwendet, bei denen die Läuse gleich beim ersten Anstich in Saugstellung verblieben.

Die Methode erlaubt es also, die Stichwirkung von Aphiden auf lebende pflanzliche Zellen in kürzester Zeit zu untersuchen und besitzt zudem den Vorteil, daß die Mundwerkzeuge als Markierung im pflanzlichen Gewebe verbleiben, wodurch die getroffene Stelle einwandfrei festgestellt werden kann.

BESTIMMUNG DES AUSBREITUNGSBEREICHES VON APHIDENSPEICHEL IN DER ZWIEBELSCHUPPE VON ALLIUM CEPA

Um die Ausschüttung und Ausbreitung des radioaktiv markierten Aphidenspeichels in Pflanzenzellen festzustellen, wurde das sog. Stripping-Filmverfahren angewendet. Zu diesem Zweck wurden in kleine Quadrate zerschnittene Zwiebelschuppen von *Allium cepa* radioaktiven Aphiden zum Anstich vorgelegt. Verwendet wurden nur Tiere, die vorher mindestens dreimal in nichtradioaktive Gewebe eingestochen hatten und sich so von evtl. äußerlich anhaftendem P^{32} befreit hatten. Außerdem durften diese Tiere keine Probesaugstiche durchführen.

Nach der vorher erwähnten Methode wurde den Aphiden der Rüssel abgeschnitten. Die Epidermisquadrate wurden anschließend abgezogen und auf einen Objektträger gebracht. Dann wurde im Dunkeln auf dieses Präparat ein Spezialfilm (Kodak, Scientific Plates, Autoradiographic) aufgetragen. Dieser Film schließt das Präparat deckglasartig nach oben ab. Nach 14-tägiger Exposition wurde entwickelt und fixiert.

Auf diese Art und Weise erhält man ein Präparat, das als Deckschicht einen entwickelten Film besitzt, unter dem sich die Zwiebelschuppe befindet. Nachdem das Epidermishäutchen mit der Emulsionsschicht verklebt, ist seine Schrumpfung während der Exposition unbedeutend. Die Teile des Filmes, die vor dem Entwickeln über radioaktiven Zellen gelegen hatten, sind geschwärzt. So ist es möglich, genauere Angaben über die Ausbreitung des Speichels in den einzelnen Zellen zu geben.

Die Auswertung der Ergebnisse zeigte folgendes: Von der Stichstelle aus gesehen breitete sich der radioaktiv markierte Speichel im allgemeinen in einer Ellipsenform aus, und zwar so, daß der größte Durchmesser des Ausbreitungsbereiches, die Hauptachse, 10 bis 20 Längsseiten der langgestreckten Zellen entsprach. Der kleinste Durchmesser, die Nebenachse, hatte maximal die halbe Länge. Zellen, die in diesem bezeichneten Bereich lagen, konnten deshalb bei den nachfolgenden Untersuchungen zum Vergleich nicht herangezogen werden. Die Vergleichszellen mußten außerhalb des Wirkungsbereiches des Läusespeichels liegen, wenn einwandfreie Ergebnisse erzielt werden sollten. Durch diese Bestimmung wurde es demnach ermöglicht, an der gleichen Schuppe Zellen des Stichbereiches mit vollkommen unversehrten Zellen zu vergleichen.

DIE WIRKUNG VON APHIDENSTICHEN AUF DIE DEPLASMOLYSEZEIT

Kloft hatte bei seinen Untersuchungen über die Wirkung von Speichel der Feuerwanze, *Pyrrhocoris apterus* L., auf die Wasserpermeabilität von *Spirogyra* sp. eine Permeabilitätssteigerung gefunden. Es lag nun die Vermutung nahe, daß Aphidenspeichel eine ähnliche Wirkung zeigt. Da es aber nicht möglich ist, Aphidenspeichel in solchen Mengen zu gewinnen, die ausreichen, um gleichartige Untersuchungen bei bestimmten Konzentrationen durchzuführen, wurde die vorher beschriebene Methode den Untersuchungen zugrunde gelegt. Auf diese Weise wurde aber gleichzeitig erreicht, daß die Versuchsbedingungen den natürlichen Einstichsbedingungen entsprachen.

Die präparierten Epidermisquadrate, in denen der Rüssel nach dem Abschneiden zur Markierung zurückgeblieben war, wurden mit einer Pinzette vorsichtig abgehoben und auf einen Objektträger in chlorfreies Leitungswasser gebracht, also in das gleiche Medium, in dem sie sich vor dem Anstich befanden. Mit Hilfe eines Phasenkontrastmikroskops wurde anschließend bei Verwendung von Ölimmersion die Lage der Stechborsten bestimmt.

Es wurde zunächst unterschieden, ob der Anstich intra- oder interzellular lag. Die meisten Aphiden hatten interzellular angestochen und dabei Stellen bevorzugt, an denen drei Zellen aneinandergrenzten.

20 Minuten nach dem Abschneiden der Stechborsten wurden die Häutchen in eine 0,8 molare KNO_3-Lösung gelegt und plasmolysiert. Direkt getroffene Zellen waren größtenteils tot, sie zeigten keine Deplasmolyse. Physikalisch-chemisch wird ja die Semipermeabilität, also praktisch die Plasmolysierbarkeit und Deplasmolysierbarkeit vakuolisierter Zellen, als Kriterium für den lebenden Zustand angesehen (PAECH & EBERHARDT, 1956).

Die Plasmolyse direkt getroffener, aber nicht getöteter Zellen wurde unter dem Mikroskop beobachtet. Die Epidermisquadrate wurden hierzu auf einen Hohlschliffobjektträger in KNO_3 gebracht. Hierbei zeigten die direkt getroffenen Zellen sofort oder zumindest schneller als die Vergleichszellen konvexe Plasmolysen. Die Kontrollzellen wiesen dagegen eine Konkavform auf, die erst nach einiger Zeit in Konvexform überging.

Auf anderen Hohlschliffobjektträgern wurde unter Zuhilfenahme eines prismatischen Zwischenstückes, welches binokularen Einblick für zwei Beobachter ermöglicht (sog. Diskussionstubus der Firma Zeiß-Winkel), die Deplasmolysezeit der Epidermiszellen in destilliertem Wasser unter dem Phasenkontrastmikroskop gemessen (MAREK & KLOFT, 1956). Es wurden miteinander verglichen die Deplasmolysezeitwerte des Stichbereiches und die Werte der Zellen, die außerhalb des Wirkungsbereiches des Speichels lagen. Letzterer wurde, wie bereits erwähnt, mit radioaktivem Speichel festgelegt. Es wurden zum Vergleich annähernd gleichgroße Zellen der gleichen Schuppe und der gleichen Region gewählt. Dies ist besonders wichtig, da in der Literatur auf die Schwierigkeit hingewiesen wird, daß sich die einzelnen Schuppen einer Zwiebel in der Deplasmolysezeit beträchtlich unterscheiden können (DRAWERT, 1954). Diese Differenzen in den Deplasmolysezeiten werden zum Teil auf die unterschiedlichen Zellgrößen zurückgeführt (DRAWERT, 1954). Die physiologischen Unterschiede sind teilweise dadurch begründet, daß die innersten Schuppen die jüngsten, die äußersten die ältesten sind. Darüber hinaus zeigen auch basaler und apikaler Teil einer Schuppe diese Verschiedenheit (STRUGGER, 1949), was ebenfalls bei meinen Vorarbeiten bestätigt werden konnte.

Aus der Vielzahl der Versuche wurden einige herausgegriffen und getrennt nach inter- und intrazellularem Anstich in Tabelle I aufgeführt. Ihre Auswertung ergibt, daß die Zellen des Stichbereiches in jedem Fall eine verkürzte Deplasmolysezeit zeigen. Es tritt demnach durch den Läusestich eine Veränderung der

TABELLE I

Wirkung von Stichen der Zwiebellaus auf die Deplasmolysezeit der Epidermiszellen von Allium cepa

A. *Intrazellularer Anstich*

Vers. Nr.	Datum	Deplasmolysezeit in Sek.		t	n	P	$\frac{K}{T}$
		Vergleichs-zellen	Getroffene Zellen				
1 (7)	15.4.55	245	170	3.967	7	$<0,01$	1,44
2 (13)*	20.4.55	140	107	3,95	4	$<0,02$	1,30
3 (30)	30.4.55	152	60	5,421	5	$<0,01$	2,53
4 (32)	7.5.55	164	67	4,955	16	$<0,001$	2,44

* Rüssel intrazellular; Abstand der beiden Rüssel 10 Zellen; in der einen angestochenen Zelle ausgebildete Speichelscheide (3/4 der Zellbreite)

B. *Interzellularer Anstich*

Vers. Nr.	Datum	Deplasmolysezeit in Sek.		t	n	P	$\frac{K}{T}$
		Vergleichs-zellen	Zellen im unmittelbaren Stichbereich				
1 (3)	15.4.55	235	155	4,818	7	$<0,01$	1,51
2 (12)	20.4.55	243	193	2,954	7	$<0,05$	1,25
3 (18)*	27.4.55	153	75 bzw. tot	4,125	12	$<0,01$	2,04
4 (21)	30.4.55	152	67	8,863	7	$<0,001$	2,26

* 1 Zelle tot

Wasseraufnahme gegenüber den Kontrollzellen auf. $\frac{K^{1)}}{T}$ ist in jedem Falle > 1, d.h. das Wasser tritt in die plasmolysierten, angestochenen Zellen schneller ein als in die plasmolysierten Vergleichszellen. Bei den Versuchen mit intrazellularem Anstich wurde, wenn die direkt getroffene Zelle nicht abgetötet war, nur deren Deplasmolysezeit angegeben.

Besonders sei auf den Versuch A 2 hingewiesen, bei dem es gelang, zwei Aphiden nebeneinander zum Anstich zu bringen. Die geschädigten Zellen hatten die gleiche Deplasmolysezeitverkürzung.

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1) K = Mittelwert der Deplasmolysezeit der Kontrollzellen
T = Mittelwert der Deplasmolysezeit der Zellen im Stichbereich
$\frac{K}{T}$ = 1 : Deplasmolysezeit der Kontrollzellen = Deplasmolysezeit der Zellen im Stichbereich
$\frac{K}{T} < 1$: Erhöhung der Deplasmolysezeit
$\frac{K}{T} > 1$: Verkürzung der Deplasmolysezeit

WEITERE WIRKUNGEN DER APHIDENSTICHE AUF DIE PFLANZLICHE ZELLE

Aus der Literatur ist bekannt, daß Traumata oder bestimmte Chemikalien Plasmaströmungen auslösen. Nachdem der Aphidenstich, wie bereits erwähnt, einen kombinierten Reiz mit einer mechanischen und chemischen Komponente darstellt, war mit einer Beeinflussung der Plasmaströmung mindestens in den getroffenen bzw. in den an die Stichstelle angrenzenden Zellen zu rechnen. Diese Vermutung sollte unter Anwendung nachfolgender Versuchsanordnung bestätigt werden:

Die innere Epidermis der Zwiebelschuppe von *Allium cepa* wurde nach der vorher beschriebenen Methode vorbehandelt. Die Stichstelle blieb durch den in der Epidermis steckenden Rüssel markiert. Die Epidermisquadrate wurden in chlorfreies Leitungswasser auf einen Objektträger gebracht und mit einem Deckglas, welches mit Glassplitterchen unterstützt war, abgedeckt. Letztere sollten ein Quetschen des Präparates verhindern. Es ist davon abzuraten, destilliertes Wasser für diese Untersuchungen zu verwenden, da dieses wegen seines Metallspurengehaltes (Cu) leicht zu oligodynamischen Schädigungen des Protoplasten führt (STRUGGER, 1949). Unter dem Phasenkontrastmikroskop konnte bei Ölimmersion mit Hilfe eines Okularmikrometers die Geschwindigkeit der Plasmaströmungen an möglichst kleinen Teilchen, die vom Plasma mitgeschleppt werden, in der getroffenen Zelle oder in den dem Anstich benachbarten Zellen gemessen und mit der Strömungsgeschwindigkeit von Zellen außerhalb des Stichbereiches verglichen werden. Nach Strugger eignen sich für diese Messungen die Mikrosomen. Gewöhnlich mißt man die Zeit, die ein Mikrosom für die Passage von 10 Teilstrecken des Okularmikrometers braucht. Zur genaueren Bestimmung wird die Geschwindigkeit mehrerer Mikrosomen gemessen (vgl. STRUGGER, 1949).

TABELLE II

Wirkung von Stichen der Zwiebellaus auf die Plasmaströmung von Allium cepa *(gemessen 30—120 Minuten nach dem Anstich)*

Vers. Nr.	Datum	Zeit für 10 Passagen in Sek.		t	n	P
		Zellen, die an die Stichstelle direkt angrenzen	Vergleich			
1	28.1.55	1,9	2.99	10.8	71	<0,001
2	7.2.55	5,3	10,4	7,127	19	<0,001
3*	8.2.55	3,4	6,8	3,415	19	<0,01
4*	9.2.55	11,4	15,3	2,47	14	<0,05
9	14.2.55	4,5	11	5,78	13	<0,001
16**	18.2.55	3,2	4,4	4,102	25	<0,001

* kleine Speichelscheide in der einen Zelle
** intrazellular angestochen

In den getroffenen bzw. in den an die Anstichstelle angrenzenden Zellen konnte nach dem Anstich eine Steigerung der Strömungsgeschwindigkeit verzeichnet werden (Tabelle II). Eine lebhafte Plasmaströmung wird von verschiedenen Autoren als ein leicht wahrnehmbares Zeichen besonderer Leistungen der Zelle angesehen oder als eine Vorbereitung zu solchen. Bei intrazellularem Anstich ging in den direkt getroffenen Zellen bei einzelnen Präparaten mehrere Stunden lang ein Plasmastrom vom Kern zur Stichstelle. Der Strom brachte zahlreiche Mikrosomen zur Stichstelle, so daß sich in ihrer Nähe eine „Mikrosomenwolke", eine Ansammlung vieler Mikrosomen, ausbildete. Häufig war es nicht möglich, die Plasmaströmung in den ersten Minuten nach dem Anstich zu messen. Sie setzte jedoch einige Zeit später ein und zeigte dann eine deutliche Erhöhung gegenüber den Vergleichszellen. Vielleicht läßt sich diese Erscheinung mit einer vorübergehenden Viskositätserhöhung in Zusammenhang bringen, denn die Strömung kann um so schwerer stattfinden, je zäher das Plasma ist (BÜNNING, 1953). Nach Bünning kann eine starke Viskositätserhöhung als Folge schädigender mechanischer Einwirkung auftreten. Die Präparate wurden, wie am Beispiel von Tab III gezeigt wird, mehrere Tage beobachtet.

TABELLE III

Wirkung von Stichen der Zwiebellaus auf die Plasmaströmung von Allium cepa *(gemessen 1—96 Std. nach dem Anstich)*

Vers. Nr.	Datum	Zeit nach dem Anstich	Zeit für 10 Passagen in Sek.		t	n	P
			Zellen, die direkt an die Stichstelle angrenzen	Vergleichszellen			
4*	7.2.55	1 Std.	3,4	6,8	3,415	19	<0,01
	8.2.55	24 Std.	11,5	11,6	0,134	25	<0,09
	9.2.55	48 Std.	14,5	11	6,456	25	<0,001
	10.2.55	72 Std.	keine Plasmaströmung meßbar	14	—	—	—
	11.2.55	96 Std.	Zellen tot	keine Plasmaströmung meßbar	—	—	—

* kleine Speichelscheide in einer Zelle

Hierbei wurde festgestellt, daß innerhalb von 24 Stunden die erhöhte Strömungsgeschwindigkeit der Zellen des Stichbereiches nach ihrer anfänglichen Steigerung nachläßt und sich der Geschwindigkeit der Vergleichszellen anpaßt, um nach einigen Stunden weiter abzusinken. Ferner konnte bei allen Versuchen nachgewiesen werden, daß die im Stichbereich liegenden Zellen früher absterben als die Vergleichszellen. Wenn auch die Mechanik der Strömungserscheinungen im Plasma ungeklärt ist, so steht es doch fest, daß die Strömung irgendwie an den energieliefernden Stoffwechsel der Zellen gebunden ist (BÜNNING, 1953) und durch den Läusestich beeinflußt wird.

Bei diesen Untersuchungen wurden ferner noch folgende Beobachtungen gemacht:1)

Bei intrazellularem Anstich waren die direkt getroffenen Zellen größtenteils tot. Das Plasma dieser Zellen zeigte eine relativ grobe, granulose Koagulationsstruktur und keine Strömung. Die Zellkerne waren dann geschrumpft, grobkörnig und daher deutlich sichtbar. Beim Einlegen in ein Plasmolytikum plasmolysierten diese

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1) Bei der Beschreibung der zytologischen Reaktionen wurde die Terminologie nach STRUGGER (1949) verwendet.

Zellen nicht. Andere direkt getroffene Zellen wiesen eine Reizplasmolyse auf, waren also plasmolysiert, ohne daß dieser Vorgang von außen osmotisch herbeigeführt wurde. ZWEIGELT (1931) hatte bereits in seinen Arbeiten diese Erscheinung bei einzelnen Aphiden festgestellt, während RAWITSCHER (1933) bei *Doralis fabae* und *Brachycaudina napelli* das Eintreten der Plasmolyse nicht beobachten konnte, aber die Möglichkeit offenließ, daß sich einzelne Blattlausarten in dieser Hinsicht verschieden verhalten (WEBER, 1933).

Waren die Stechborsten in die Zelle eingedrungen, so konnten häufig Speichelscheiden beobachtet werden, die im Mikroskop stark lichtbrechend erscheinen Derartige Speichelscheiden werden auch in Zuckerlösungen ausgebildet, die man Blattläusen durch eine Kollodiummembran anbietet (SCHMIDT, 1959).

Wie die Untersuchungen von VAN HOOF (1958) zeigen, ist jedoch die Speichelscheide in der Zellwand anders als im Zellumen und in der Flüssigkeit ausgebildet. Während die Speichelscheide in der Zellwand einen regelrechten Kanal von gleichweitem Durchmesser darstellt, in dessen Zentrum die Bahn für die Stechborsten liegt (VAN HOOF, 1958), besitzt sie in der Zelle (vgl. Abb. 2 und 3) und in der Flüssigkeit ein perlschnurartiges Aussehen (SCHMIDT, 1959). Die Speichelscheide baut sich aus einer Vielzahl von Tröpfchen auf, da jedes Verschieben der Stechborsten mit einem Ausstoß von Speichelflüssigkeit verbunden ist, die die Stechborstenspitze umschließt und gallertig fest wird (SCHMIDT, 1959). Nach dem Herausziehen der Stechborsten ist auch hier ein zentraler Kanal deutlich erkennbar (vgl. Abb. 2, SCHMIDT, 1959). Verzweigte Speichelscheiden, wie sie von VAN HOOF (1958) und von SCHMIDT (1959) beobachtet wurden, konnten von mir in den Epidermiszellen von *Allium cepa* nicht festgestellt werden.

Bei intrazellularem Anstich waren die der Stichstelle benachbarten Zellen immer lebend, was vor allen Dingen durch das starke Hinfließen der Mikrosomen in Richtung der Stichstelle bewiesen werden konnte. Bisweilen trat in einer an die Stichstelle angrenzenden Zelle eine Kreisströmung auf, deren Durchmesser kleiner als der Kerndurchmesser war. Ein Hinwandern der Kerne in Richtung der Stichstellen konnte von mir nicht eindeutig festgestellt werden. Ich möchte dies auf die relativ geringe Menge Speichel, die kurze Saugdauer und die Feinheit der Stechborsten zurückführen. Bei einzelnen Versuchen konnte jedoch eine Kernschrumpfung nachgewiesen werden. Da Tabelle III zeigt, daß die Zellen im Stichbereich früher absterben, dürfte sich hier wohl eine Kernpyknose anbahnen.

Auf Grund der Untersuchungen lassen sich die Vorgänge beim Eindringen der Aphidenstechborsten in die Pflanzenzelle folgendermaßen zusammenfassen:

Haben die Aphiden mit ihren Stechborsten die Zellwand durchstoßen, so gelangen die Stechborsten in das Innere der Zelle. Dieses Durchbohren stellt einen mechanischen Prozeß dar und wird deutlich durch die auf- und niedergehende Bewegung der Zellwand; diese Bewegung wird durch den Druck der Stechborsten hervorgerufen (VAN HOOF, 1958). Die Laus sondert, wenn auch nur in geringen Mengen, Speichel ab, der auf das Plasma wirkt bzw. zum Aufbau der Speichelscheide verwendet wird. Manchmal genügt diese Speichelmenge zur Abtötung der Zelle. Im Modellversuch läßt sich der gleiche Effekt nur dann mit Nadeln erzielen, wenn diese die vier- bis zehnfache Stärke der Stechborsten haben. Es müssen sich also im Speichel Stoffe befinden, die auf das Plasma toxisch wirken, denn die relativ geringe mechanische Verletzung kann allein für das Abtöten der Zellen nicht verantwortlich gemacht werden. Hier dürften Unterschiede zwischen dem Speichel der einzelnen im Phloem saugenden Pflanzenlausarten bestehen, da z.B. bei *Pseudococcus*-Arten die Stechborsten die Zellen ohne tiefergehende Schädigung durchbohren können. Inwieweit die von MILES (1959) angenommenen verschiedenen Fraktionen des Speichels hier unterschiedliche Wirkung nach sich ziehen, ist noch nicht untersucht.

Kommen die Stechborsten in den peripheren Zellbereich, so rufen sie hier Schädigungen hervor. Nach den Untersuchungen von LEPESCHKIN (1924) an *Spirogyra* sind die oberflächlichen Schichten des Protoplasmas aber viel empfindlicher gegen mechanische Reize als dessen innere Schichten und werden permeabel für gelöste Stoffe. Als weitere Folgeerscheinung tritt in der Zelle eine lebhafte Plasmaströmung auf. Die Dauer dieser Vorgänge steht in enger Beziehung zur Reizstärke.

DIE ZELLPHYSIOLOGISCHE WIRKUNG DER MECHANISCHEN KOMPONENTE DES APHIDENSTICHES

Für den Nachweis der zellphysiologischen Wirkung der mechanischen Komponente des Aphidenstiches war es notwendig, Nadeln herzustellen, die die Stärke des Stechborstenbündels hatten. Die Herstellung solcher Nadeln erfolgte durch galvanische Ätzung von Insektennadeln der Stärke 000 nach BURKHARDT (1954). Der Spitzendurchmesser dieser Nadeln beträgt einige μ, da sich die geätzten Nadeln zur Spitze sehr schlank verjüngen (BURKHARDT, 1954). Um zu verhindern, daß beim Eindringen der feinen Stahlnadeln eine Reaktion des Metalls mit dem Zellsaft stattfindet, wurden die Nadelspitzen mit Araldit-Lack (Herbol V 8394) überzogen. Die so behandelten Nadeln wurden bei höherer Temperatur getrocknet. Stärkere Nadeln rufen auch stärkere Verletzungen hervor und können deshalb zum direkten Vergleich dieser Untersuchungen nicht herangezogen werden.

Zunächst sollte die Wirkung des mechanischen Reizes auf die Deplasmolysezeit von Zellen der Epidermisschuppe von *Allium cepa* untersucht werden. Die nach der vorher beschriebenen Methode präparierten Zwiebelschuppen wurden unter dem Binokular angestochen. Der Stich mußte sehr sorgfältig durchgeführt werden, da sonst zu viele Zellen verletzt worden wären. Am besten war es, die abgeätzte Nadelspitze in einer Länge von 1 mm von der Nadel abzutrennen und mit einer feinen Pinzette in das pflanzliche Gewebe einzuführen. Die Nadelspitze diente dann — wie zuvor der Rüssel — als Markierung. Da von der Nadelspitze nur der vorderste Teil die Stärke der Stechborsten besaß, durfte die Nadel nicht zu tief in das Gewebe eingeführt werden.

Vergleichsweise wurde dieser Versuch mit feinsten Kapillaren durchgeführt, die jedoch niemals die Feinheit der Nadeln erreichten. Glaswollfäden konnten wegen ihrer Elastizität die Zellwand nicht durchstoßen. Nach dem Anstich wurden die Zellen, wie bei den vorhergehenden Versuchen, in 0,8 molarer KNO_3-Lösung plasmolysiert und in Aqua dest. deplasmolysiert.

Der Stichbereich zeigt auch hier (Tabelle IV) eine deutliche Verkürzung der Deplasmolysezeit. Eine Veränderung in der Wasseraufnahme wird also bereits

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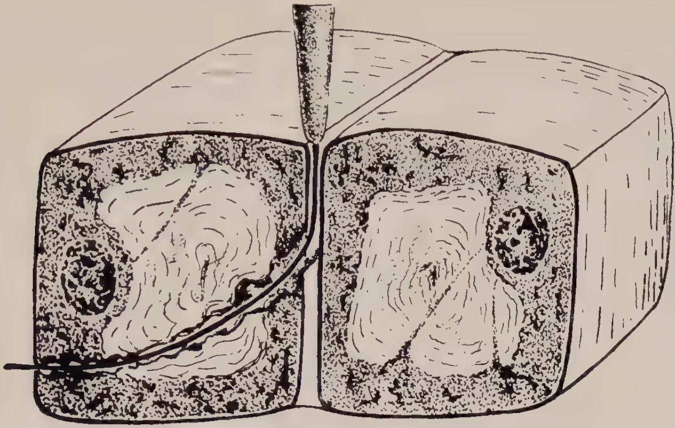

Abb. 2. Epidermiszellen von *Allium cepa* mit eingesenkten Stechborsten (schematisiert)

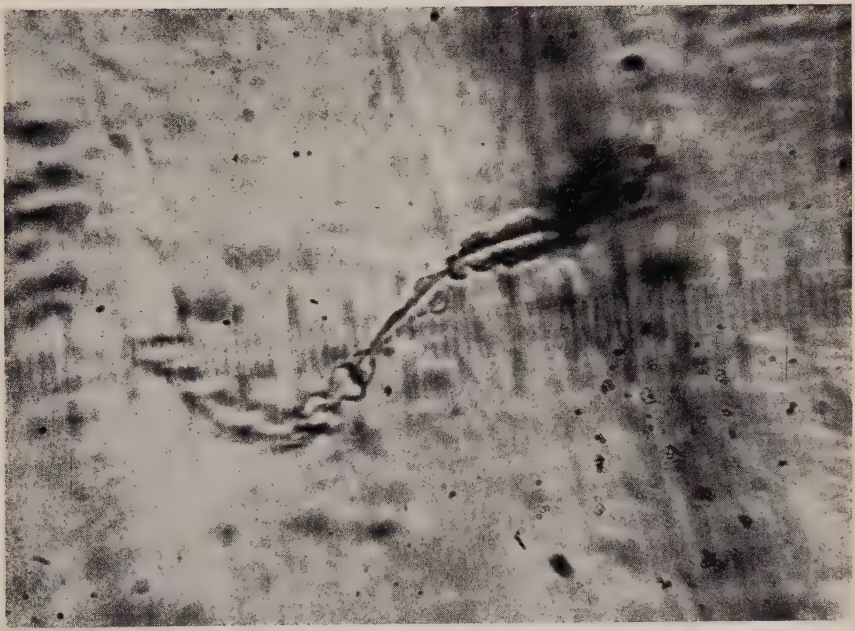

Abb. 3. Speichelscheide (stechborstenfrei) eines Suchstiches in einer Zelle von *Allium cepa*, 10 Minuten nach dem Einstich im Phasenkontrastmikroskop gesehen

durch den mechanischen Reiz — durch das Eindringen der Stechborsten — erreicht. Wurden die Nadelspitzen zu tief in das Gewebe eingesenkt, so waren die getroffenen Zellen, teilweise auch die angrenzenden Zellen, tot. Die Zellwände wiesen zum Teil starke Verletzungen auf. Aber auch hier zeigte die Umgebung der verletzten Zellen eine Verkürzung der Deplasmolysezeit. In den der Stichstelle benachbarten Zellen konnte außerdem eine lebhafte Plasmaströmung wahrgenommen werden, die viele Mikrosomen heranbrachte. Es liegt die Vermutung nahe, daß die Zelle durch deren Heranschaffen etwaige Schädigungen auszugleichen versucht. Eine einwandfreie Messung der Strömungsgeschwindigkeit von lebenden, direkt getroffenen Zellen war bei Ölimmersion nicht möglich, da beim Auflegen der Deckgläschen die Nadelspitze aus der Zelle herausgedrückt wurde, diese dann verdeckte oder sich weiter eindrückte und stärkere Schädigungen hervorrief. Die Nadel zeigt ja in keiner Weise die gleiche Elastizität wie der abgeschnittene Rüssel.

TABELLE IV

Wirkung von Nadelstichen auf die Deplasmolysezeit der Epidermisschuppe von Allium cepa

Nr. Vers.	Datum	Deplasmolysezeit in Sek. Vergleichszellen	Deplasmolysezeit in Sek. Zellen im unmittelbaren Stichbereich	t	n	P	$\frac{K}{T}$
1 (3)	23.3.56	133	73	5,66	10	$<0{,}001$	1,82
2 (7)	23.3.56	183	89	3,857	10	$<0{,}01$	2,05
3 (8)	24.3.56	119	39	5,201	9	$<0{,}001$	3,05
4 (31)*	29.3.56	84	24	9,169	11	$<0{,}001$	3,5

* intrazellular getroffene Zelle tot

Bei einigen Versuchen gelang es, den Einstich der Nadelspitze so zu lenken, daß die Anstichstelle dicht neben der Zellwand lag. Dabei zeigte die getroffene Zelle eine Reizplasmolyse. KÜSTER (1956) berichtet uns hierzu, daß unter der Einwirkung mechanischer und anderer Reize das Protoplasma sich oft mit beträchtlicher Geschwindigkeit kontrahiert, ohne daß wir zu sagen vermöchten, welche Faktoren die Exosmose des Wassers veranlassen. Ungeklärt ist die Frage, ob bei dieser Reizplasmolyse das Plasma reines Wasser oder Zellsaft exosmieren läßt. Nachdem wir vorher eine Veränderung der Wasseraufnahme als Wirkung des mechanischen Reizes kennengelernt haben, kann mindestens ein teilweises Entweichen des Zellsaftes angenommen werden. Die gleiche Vermutung spricht bereits KÜSTER (1951, 1956) aus.

Beim Einlegen in ein Plasmolytikum zeigten die Zellen im Stichbereich in kürzester Zeit konvexe Plasmolyse. Strugger beschreibt uns die gleiche Erscheinung bei Zellen eines Wundrandes. Konvexe Plasmolysen deuten auf eine geringe Viskosität hin. Trotzdem sei hier darauf hingewiesen, daß aus den Plasmolyseformen nur mit großer Zurückhaltung auf die Viskosität geschlossen werden darf (KÜSTER, 1956).

Aus diesen Versuchen geht hervor, daß beim Eindringen feinster Nadeln bereits deutlich wahrnehmbare Schädigungen der Zelle auftreten.

Wenn auch die Beobachtung gemacht werden konnte, daß der mechanische Reiz feinster Nadeln nicht die weite Ausdehnung besitzt wie der intrazellulare Anstich einer Aphide, so darf seine Bedeutung doch keineswegs unterschätzt werden.

DIE ZELLPHYSIOLOGISCHE WIRKUNG DER CHEMISCHEN KOMPONENTE DES APHIDENSTICHES

Meine Untersuchungen über das Einstich- und Saugverhalten von *Myzus ascalonicus* haben gezeigt, daß die Aphiden beim Einstechen in vorgelegte Medien Speichel abgeben (MAREK, 1959). Die Ausbreitung von radioaktivem Speichel und die Ausbildung von Speichelscheiden in pflanzlichen Zellen bilden einen weiteren Beweis für die Abgabe von Speichel. KLOFT (1956) hat bereits den Aphidenspeichel analysiert und hierbei 4 Aminosäuren (Glykokoll, Alanin, Asparagin- und Glutaminsäure) gefunden. Nachdem FITTING (siehe BÜNNING, 1953) durch seine umfangreichen Untersuchungen nachgewiesen hat, daß gewisse Aminosäuren schon in geringsten Konzentrationen Plasmaströmungen auslösen, liegt der Schluß nahe, daß die verstärkte Plasmaströmung in den angestochenen bzw. der Stichstelle benachbarten Zellen auf diese Aminosäuren zurückzuführen ist. Auch das Auftreten von verstärkten Plasmaströmungen in mechanisch geschädigten Zellen (Wundreiz) läßt sich durch Aminosäuren erklären, da bei Verwundung durch den Abbau von Eiweißkörpern derartige Verbindungen frei werden (BÜNNING, 1953).

Es bleibt nur noch die Frage offen, ob reine Aminosäuren Auswirkungen auf die Deplasmolysezeit der Pflanzenzelle besitzen. Aus diesem Grunde wurden präparierte Epidermisquadrate 10—30 Minuten in Aminosäuren verschiedener Konzentrationen gelegt, anschließend plasmolysiert und die Deplasmolysezeit in Aqua dest. gemessen. Epidermisquadrate der gleichen Schuppenregion dienten als Vergleich. Bei allen diesen Versuchen konnte jedoch keine Veränderung in der Wasseraufnahme nachgewiesen werden. Reine Aminosäurelösungen scheinen demnach keinen Einfluß auf die Wasserpermeabilität zu besitzen, beeinflussen aber die Plasmaströmung.

In Fortführung dieser Untersuchungen sollte nun versucht werden, den Stich von Pflanzensaugern künstlich nachzuahmen. Zu diesem Zweck wurden in die Epidermiszellen feinste Kapillaren — Injektionsnadeln waren viel zu groß — eingeführt und durch diese die 4 vorher genannten, im Aphidenspeichel gefundenen Aminosäuren einzeln und im Gemisch eingepreßt. Die Kapillaren wurden möglichst kurz über dem pflanzlichen Gewebe abgeschnitten, so daß die getroffenen Zellen markiert blieben.

Die Methode hatte jedoch den Nachteil, daß mit Kapillaren gearbeitet werden mußte, die einen viel größeren Durchmesser als die Aphidenstechborsten hatten, und daß bei diesen Versuchen der Stich von größeren Pflanzensaugern als den Aphiden nachgeahmt wurde. Die Stärke des Stechborstenbündels beträgt bei *My-*

zus ascalonicus ungefähr 3 μ, während die verwendeten Kapillaren den 30 bis 50-fachen Durchmesser aufwiesen. Die mechanische Wirkung der Kapillaren war demnach bedeutend stärker als die von normalen Aphidenstichen. Es wurden jeweils zwei Zellen bei diesem nachgeahmten Stich getroffen und hierbei die dazwischenliegende Zellwand zerstört. Beim Einpressen der Aminosäuren in die getroffenen Zellen kam es häufig an der Stichstelle zu einer kleinen Tropfenbildung. Die Auswertung dieser Versuchsreihe ergibt wiederum eine Verkürzung der Deplasmolysezeit, also eine Veränderung in der Wasseraufnahme (Tabelle V). Die vorhergehenden Ergebnisse werden damit erhärtet.

TABELLE V

Die Wirkung des künstlich nachgeahmten Aphidenstiches auf die Deplasmolysezeit (Kapillare mit Aminosäuren gefüllt)

Deplasmolysezeit in Sek.					
Vergleichszellen	Zellen im Stichbereich	t	n	P	$\frac{K}{T}$
162	81	2,517	9	<0,05	2,0
141	35	5,66	11	<0,001	4,02
187	70	4,372	9	<0,01	2,67

BESPRECHUNG DER ERGEBNISSE

Die vorliegenden Versuche haben gezeigt, daß durch den Aphidenstich in der pflanzlichen Zelle verstärkte Plasmaströmungen und Deplasmolysezeitverkürzungen auftreten. Diese Wirkungen auf die pflanzliche Zelle können auf die zwei Komponenten des Aphidenstiches, den mechanischen und den chemischen Reiz, zurückgeführt werden. Die chemische Komponente des Aphidenstiches bildet der Speichel. Miles (1959) nimmt bei den Aphiden ähnlich wie bei den Heteroptera zwei verschiedene Speichelfraktionen an. Die eine Fraktion stellt das Material dar, aus dem die Speichelscheide gebildet wird — die Ausbildung von Speichelscheiden konnte von mir in den Epidermiszellen von *Allium cepa* häufig beobachtet werden —, die andere Absonderung ist wässeriger Speichel. Für die verstärkten Plasmaströmungen können die im Läusespeichel vorhandenen Aminosäuren bzw die bei der Verwundung durch den Abbau von Eiweißkörpern gebildeten Aminosäuren (Bünning, 1953) als Ursache angesehen werden. Fitting (siehe Bünning, 1953) hat bereits durch seine Untersuchungen nachgewiesen, daß gewisse Aminosäuren schon in geringsten Konzentrationen Plasmaströmungen auslösen.

Bei getroffenen Epidermiszellen von *Allium cepa* konnte eine Verkürzung der Deplasmolysezeit gegenüber den Kontrollzellen gefunden werden. Es kommt demnach durch den Läusestich zu einer Veränderung in der Wasseraufnahme, die sich in einem schnelleren Eintreten des Wassers in die getroffene Zelle äußert und mit Hilfe des Deplasmolysetestes erfaßt werden kann. Jede Zelle besitzt bereits im unplasmolysierten Zustand neben dem osmotisch gebundenen Wasser noch eine zusätzliche, nichtosmotisch in der Vakuole festgehaltene, Wassermenge, die bis zu 22% des gesamten Zellsaftes ausmachen kann, aber der Vakuole mit bloßen osmotischen Mitteln nicht entzogen werden kann (Bogen & Follmann, 1955). Die nichtosmotische Aufnahme dieses Wassers in die Zelle erfordert eine zusätzliche Energieleistung von seiten der Zelle und ist daher mit der Gesamtenergiebilanz der Zelle gekoppelt (BOGEN & PRELL, 1953; BOGEN, 1953). Eine Beeinflussung dieser Prozesse ist auf dem Wege über Stoffwechselgifte möglich (BOGEN & FOLLMANN, 1955). Es ist daher auch denkbar, daß Inhaltsstoffe des Pflanzenlausspeichels, die nach den vorliegenden Untersuchungen den pflanzlichen Stoffwechsel verändern, indirekt auf dem Wege über solche Stoffwechseleffekte auch die nichtosmotische Wasseraufnahme und Wasserabgabe beeinflussen.

Wundreize scheinen die gleiche Wirkung auf die Wasseraufnahme auszuüben, da beim Anstechen der Zellen mit feinsten Nadeln ebenfalls eine Veränderung der Deplasmolysezeit nachgewiesen werden konnte. Die mechanische Komponente wurde bei den bisherigen Untersuchungen im allgemeinen vernachlässigt, da man die Verletzungen, die durch die feinen Aphidenstechborsten hervorgerufen werden, für unbedeutend hielt. Die zellphysiologischen Untersuchungen haben jedoch die Bedeutung solcher geringfügiger Verletzungen aufgezeigt. Auch mit den feinsten Nadelspitzen durchgeführte Anstiche verursachen in der Zelle Schädigungen, die sich erfassen lassen.

Der künstlich nachgeahmte Läusestich (durchgeführt mit Aminosäuren enthaltenden feinen Glaskapillaren) hatte den Nachteil, daß sicher nicht alle Speichelbestandteile in die Zelle eingebracht werden konnten. Es ist keineswegs anzunehmen, daß die im Speichel unseres Versuchstieres bisher gefundenen 4 Aminosäuren die einzigen wirksamen Bestandteile des Speichels sind. Vielmehr muß damit gerechnet werden, daß sich im Speichel noch Stoffe — wenn auch nur in Spuren — befinden, die auf das pflanzliche Zellplasma toxisch wirken. Bestätigt wird diese Annahme durch die Beobachtung, daß sich bei geringen Verletzungen, hervorgerufen durch Aphidenstiche, stärkere Wirkungen zeigten als bei künstlich nach geahmten Stichen.

Der Vorteil der angewandten zellphysiologischen Methode lag darin, daß die Beobachtungen am lebenden Material durchgeführt werden konnten. Weniger günstig zeigte sich aber bei der Auswertung der Umstand, daß das Versuchstier, die Zwiebellaus, *Myzus ascalonicus*, ein Siebröhrensauger ist, während das vorgelegte pflanzliche Gewebe parenchymatischer Natur war. Bisher ist es aber nicht gelungen, gleichartige Versuche mit Parenchymsaugern durchzuführen, da diese ihre Saugstellen meist nur bei Häutungen wechseln. Gerade bei Parenchymsaugern wären jedoch solche Untersuchungen von besonderem Interesse. Diese Pflanzensauger sind nämlich in einer ganz besonderen Weise darauf spezialisiert, bei vorwiegend interzellularem Anstich den Geweben ihre Nährstoffe zu entziehen. Ihrem wohl fermentativ reicher ausgestatteten Speichel ist die Aufgabe gestellt, die dauernde Nahrungsaufnahme aus den umliegenden Zellen durch eine entsprechende Beeinflussung derselben zu ermöglichen. Dabei dürfte eine Permeabilitätsveränderung des Plasmas von ganz besonderer Bedeutung sein. Bis jetzt ist es noch nicht möglich gewesen, experimentell zu entscheiden, ob der Speichel parenchymsaugender Insekten auf das pflanzliche Zytoplasma toxischer wirkt als der von Siebröhrensaugern. Bei letzteren zeigten sich im Falle gelegentlicher intrazellularer Stiche im *Allium*-Epidermis-Test starke toxische Effekte, die beim normalen Anstich in die Siebröhrenzelle einstweilen nicht genauer bekannt sind. Es bleibt zu hoffen, daß noch Möglichkeiten gefunden werden, die erarbeiteten Methoden auf das vertiefte Studium parenchymsaugender Pflanzenläuse auszudehnen.

Die Arbeit ist durchgeführt mit Unterstützung der Deutschen Forschungsgemeinschaft. Zu besonderem Dank bin ich für Förderung der Arbeit Herrn Professor Dr. K. Gößwald und Herrn Privat-Dozenten Dr. W. Kloft verpflichtet.

SUMMARY

EFFECTS OF PIERCING PLANTS-CELLS BY APHIDS

The result of puncturing plant-cells by aphids was demonstrated as follows: The aphids, *Myzus ascalonicus* Donc., were allowed to pierce detached bulb scales of *Allium cepa*, in green light, and were then anaesthetized with CO_2. The stylets were then severed using iridectomy scissors (Fig. 1). The epidermis was dissected off, mounted on a slide and observed under a phase-contrast microscope. The point of injection remained marked by the stylets which stuck in the epidermis. Intercellular and extracellular puncturing could be distinguished.

It was possible to demonstrate a shortening of the time deplasmolysis in the cells lying close to the injection, and also an increase of streaming in the cell plasma. In cells which had been pierced directly, a saliva-sheath was formed (Fig. 2 and 3). The extent of the effect of the saliva was studied using radioactive aphids. After these radioactive aphids had pierced the epidermis the part containing the stylets was mounted on a slide. This preparation was kept in the dark for 14 days, with a special film (Kodak, Scientific Plates, Auto radiographic) covering it. The film was developed and fixed, and a preparation was thus obtained with the developed film covering the epidermis. Darkened spots on the film showed the parts of the cells which had been made radioactive by the saliva.

Very fine needles made by galvanic etching were also stuck into the cells, to demonstrate the mechanical effects of aphid punctures. This mechanical stimulus by needles only a few μ in diameter caused streaming in the cell plasma and a shortening of deplasmolysis time. It is clear that the importance of these mechanical effects must not be underestimated. The increased plasma-streaming was apparently caused by amino-acids in aphid's saliva, and also by the amino-acids produced from proteins when the cells were injured. KLOFT (1956) found 4 amino-acids in the saliva of *M. ascalonicus*. The effect of these amino-acids on deplasmolysis-time is being examined, but as yet no effect has been detected.

Finally, when fine glass-capillaries are used to imitate piercing by plantsucking insects and when the glass-capillaries contain amino-acids, deplasmolysis-time is reduced.

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THE EFFECT OF PRECIPITATION ON THE BREAK OF DIAPAUSE IN THE WHITE RICE BORER *RUPELA ALBINELLA* (Cr.) IN SURINAM (SOUTH AMERICA)

BY

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Diapause was studied in the white rice borer to find a sound basis for an effective rice stubble destruction programme. Diapausing larvae of this borer remain in the stubble after harvesting and carry the species over a period during which the hostplant may be scarce or absent. The influence of rainfall on the duration of diapause was experimentally analysed. As a rule the higher the amount of precipitation, the sooner the moths make their appearance. This break of diapause is accelerated the longer the diapausing larvae stayed in the dry stubble. It is suggested that under the prevailing weather conditions stubble destruction within one month after harvest will prevent the appearance of the moths.

Rupela albinella (Cr.), a white satin-like moth of the family Schoenobiidae, is a very common rice borer in Surinam. Although the crop losses caused by this borer are moderate, the discovery of a good method of control for keeping the insect in check is of economic value. For this purpose investigations were made in the 6000 hectares "Wageningen" rice area of the Foundation for the Development of Mechanized Agriculture during 1958/'59. Here two rice crops are grown annually. As the effect of chemical control turned out to be poor, special attention was paid to stubble destruction. It was known already that *Rupela* larvae remain in the stubble after harvesting in a diapausing condition, thus carrying the species over a period during which the hostplant may be scarce or absent. Before dealing with the results of research into the diapause phenomenon, that form a base for an effective stubble destruction programme, a few notes on the biology of the borer are given here.

Eggs are laid in clusters on the hostplant, usually on the undersurface of the leaves. Hatching occurs after 8—9 days. Within a couple of hours the young larva moves down a leafblade to the leaf axil. It soon bores into the leaf sheath, tunnels downwards, penetrates into the node and finally arrives in the central, hollow part of the internode. Incidentally the larva may also reach the stalk interior by tunneling perpendicularly through the stalk wall at a short distance above the node. Development to maturity takes place within one internode, generally in the third or fourth internode from the base. As a rule only one *Rupela* borer can be found in an infested rice stalk. The larval period, which comprises five ecdyses, varies from 28—36 days. Pupation takes place inside the internode, in a white, flimsy, silken cocoon. The pupal stage lasts from 10 to 13 days.

DIAPAUSE

The duration of a larval period of 28—36 days almost exclusively concerns those borers that develop in growing rice plants that have not reached the flowering stage. From this moment until ear maturity on the average only one percent of the larvae present pupate. Several of the other larvae gradually enter into diapause. Table I gives the results of a stalk analysis, carried out 1—7 days before harvesting. About one month after harvesting all larvae that remained in the stubble are diapausing. Larvae in this condition are immobile, more rounded in form and cream coloured. They are enclosed in a cylindrical, paper-like envelope, which is attached at one end to the nodal tissue at the upper- or lower side of the internode. When removed from its envelope and placed in a fresh piece of rice stalk the larva spins a new envelope and renews its diapause. Each time an envelope is damaged the larva repairs it.

TABLE I

Stalk analysis of twenty rice fields, 1—7 days before harvest (September 1958)

Field no.	Rupela present				Rupela moths emerged (empty cocoons)	Number of stalks examined	% Total infestation
	total	%feeding larvae	%(pre)pupae in cocoon	%larvae in envelope (entering diapause)			
1	270	87	2	11	35	1397	21.8
2	295	88	0	12	125	1420	29.6
3	133	91	2	7	76	1166	17.9
4	45	87	0	13	80	860	14.5
5	235	86	1	13	81	1237	25.5
6	50	98	2	0	46	1300	7.4
7	238	97	1	2	105	1200	28.6
8	72	86	0	14	50	1008	12.1
9	90	95	1	4	5	1487	6.4
10	125	94	2	4	45	1348	12.6
11	374	98	0	2	86	1800	25.6
12	150	77	3	19	121	1273	21.3
13	80	94	0	6	8	1426	6.2
14	189	95	2	3	70	1270	20.4
15	241	92	1	7	94	1390	24.1
16	130	85	0	15	72	1325	15.2
17	68	96	0	4	70	1232	11.2
18	248	93	1	6	126	1335	28.0
19	235	95	0	5	105	1665	20.4
20	106	96	1	3	145	1414	17.8
	mean:	91.5	1.0	7.5			

Diapause in *Rupela* larvae starts independently of seasonal climatic changes, either in humid or under rather dry weather conditions. It was VAN DER GOOT who already found in 1925 that the diapause in *Scirpophaga innotata* Wlk., a serious pest of rice in Indonesia, could be induced by feeding the larvae with paddy in the pre-flowering stage or in a later stage of development, irrespective of the time of the year. Several other researchers in studying various lepidopterous borer pests, e.g. SQUIRE (1937, 1940) working in the Lesser Antilles on the pink bollworm of cotton (*Platyedra gossypiella* (Saund.)), also showed that the diapause in these insects was only induced by dry and/or rich food towards maturity of the crop, i.e. by food of low moisture content. FIFE (1949), who studied the diapause in the pink bollworm in Puerto Rico, once again concludes that any factor that limits the moisture content of the insect's food, tends to induce the development of the diapause.

In Surinam *R. albinella* ends its diapause under the influence of rainfall, a normal phenomenon, as the termination of diapause in many tropical insects is brought about by moisture. The resting larva becomes active, spins a cocoon inside the envelope and pupates. This breaking of diapause by rain is undoubtedly connected with an increase of the water-content of the larva. Very probably it is the result of absorption of water vapour from the air, or of absorption through the cuticle of water that might have soaked the envelope to some extent. The chance of restoring the water-balance to a higher level by actual drinking (vide MELLANBY & FRENCH, 1958) will be small, as the envelope forms a good water barrier.

CORRELATION BETWEEN RAINFALL AND DURATION OF DIAPAUSE

Method

The duration of the diapause in *Rupela* in relation to rainfall was studied in a showering experiment lasting from December 1958 until August 1959. Stubbles, present in a field which was known to be heavily infested by *Rupela*, were collected 3 weeks after harvesting, at the end of October.

After having been cut to a uniform length, measuring 50 cm from the rootbase to the top of the stalk, they were stored in wooden boxes (55 × 30 × 20 cm) in a number of 300 stalks per box. Each had a small-mesh gauze bottom, a detachable gauze cover and a pair of screened slits in both longitudinal walls. This construction gave a good access of air. During the experiment the boxes were kept on racks in a field insectary, a large open screened cage (5 × 5 × 3 metres) with a roof that gave protection from rains. The boxes were grouped in four series, each series being composed of 7 boxes. The experiment on the first series started on December 1st, that on the other three at 30 days intervals after this date. Each series covered a period of 150 days. The stalks were taken out of the boxes, spread evenly on a wire netting tray of the size of 1.5 sq.m., and uniformly wetted. So 30 litres of water, corresponding to a precipitation quantity of 20 mm, were applied in 16—18 minutes per tray. After showering the stalks were kept on the trays until they regained their normal air-dry colour. This period varied from 3—20 hours, depending on the relative humidity of the air, the amount of sunshine and the wind velocity. Normally the wetted stalks were dry after 5—6 hours.

The following system of showering by means of a watering can was carried out. The stubbles of the first box of each series received 20 mm of precipitation every other day, those of the 2nd, 3rd, 4th, 5th and 6th box were wetted by this quantity every fourth, sixth, eighth, tenth and fourteenth day respectively. These treatments correspond with a monthly precipitation of 300, 150, 100, 75, 60 and 40 mm. The seventh box was left untreated.

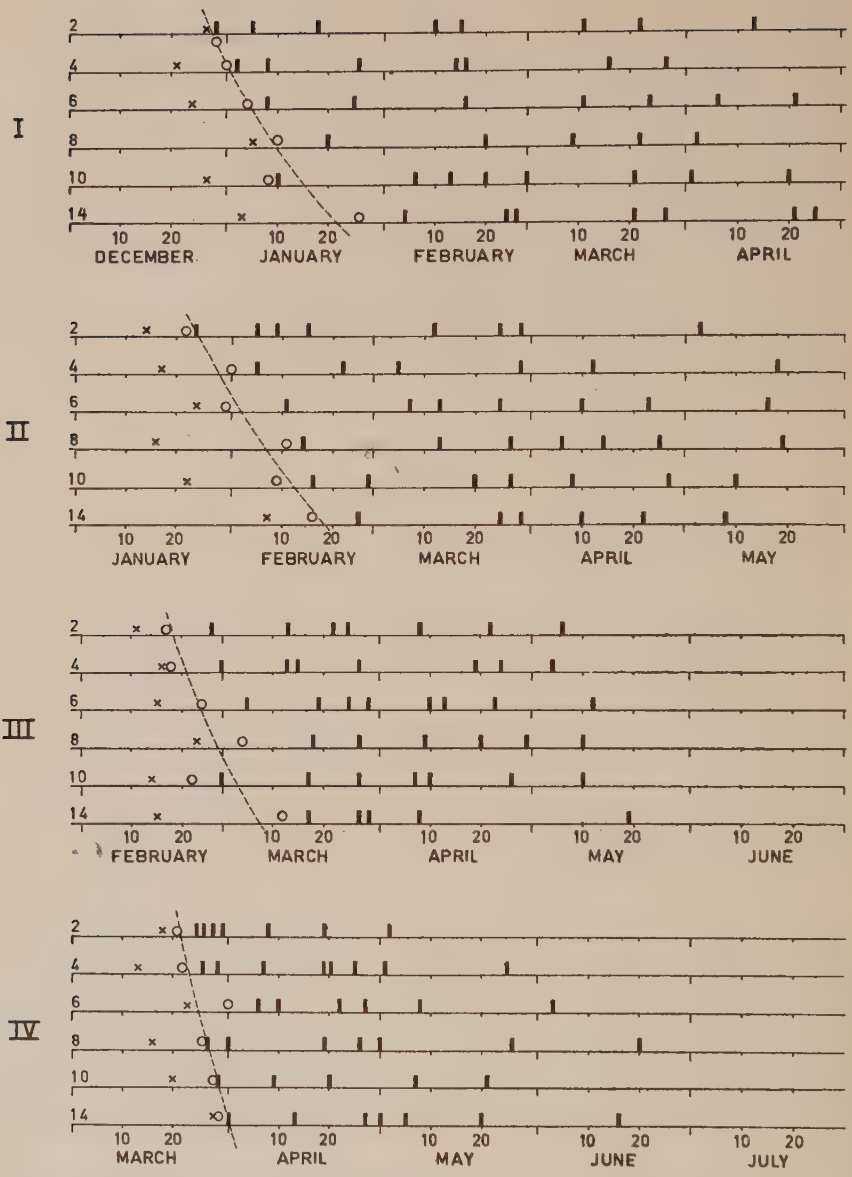

Fig. 1. Moths' emergence (expressed in groups of 10) from diapausing larvae in rice stubble after showering. The data refer to four test series of six units, each unit containing 300 stalks. Showering of the series started at 30 days intervals. The first unit of each series received 20 mm of precipitation every other day, the other units received this amount every 4th, 6th, 8th, 10th and 14th day, respectively. Each series covers a period of 150 days. x = emergence date of first moth; o = "five moth"-level.

Results

The results from the daily counts of the emerged moths in the treated boxes are summarized in fig. 1. Here the moths are listed in groups of ten. Moreover, for each treatment the date of appearance of the first moth as well as the "5-moth level" have been noted. The last mentioned level is only introduced to indicate that the emergence of moths is in full swing. At the end of the experimental duration of each series the stubbles in the boxes were cut open and examined for borers. In the treated stalks on the average 0.3—2.3% of the number of diapausing larvae originally present, were observed; the higher percentages were found in the stubbles treated every 8th, 10th and 14th day.

During the showering experiment a very small number of moths emerged from the untreated stalks. This is probably due to the effect of the October rainfall, that wetted the stubble before the date of collecting. During the 3 week's period after harvesting, when the stubbles were still in the field, the total rainfall amounted to 43 mm. This quantity was mainly produced by a shower of 30 mm, registered on October 20th. As the number of emerged moths from the untreated stalks only formed 2—4% of the number of moths that emerged from the treated ones, the influence of the October rainfall on the showering experiment is negligible.

CONCLUSIONS

From the foregoing data the following may concluded:

(1) the higher the amount of precipitation the sooner the first moths as a rule make their appearance.

(2) the break-up of diapause caused by showering is accelerated, as the diapausing larvae previously stayed longer in the dry stubble.

(3) the emergence of the vast majority of moths is completed within 1½—3 months after the appearance of the first moths; the longer the diapausing larvae previously had stayed in the dry stubble, the shorter this period is.

In an additional small-scale experiment pieces of rice stubble, containing some scores of diapausing larvae, were collected on April 3rd, 1958, and stored in the insectary. On April 1st, 1959, the larvae were still alive, though slightly decreased in size. Wetting the pieces of stubble every other day from this date on did not result, however, in pupation. By the end of April all the larvae had succumbed. From this it might be concluded that, although the diapausing stage can be prolonged to more than one year, there is a limit beyond which a further normal development cannot be achieved. It is clear now that this moment will be found somewhere between 8 and 13 months.

STUBBLE DESTRUCTION AS A CONTROL MEASURE

Up to the present rice is the only known foodplant of *R. albinella.* Notwithstanding the search for alternate hosts no other hostplant has been found. Once *Rupela* larvae were observed boring in *Luziola spruceana* Benth., a rather common wild grass growing in humid habitats. It is, however, not certain which of the five *Rupela* species present in Surinam was involved.

Although it is rather probable that a wild host exists, the main source of infestation for every new rice crop undoubtedly originates from rice plants already infested in the previously harvested crop. This means that an intensive stubble destruction in the Wageningen rice polder — an enclave in the midst of tropical rain forests and swampy areas — forms a useful control measure. In practice the stubble is flattened by weed cutters and then burned.

To evaluate the data obtained by the showering experiment (fig. 1) in relation to normal field conditions the monthly means for rainfall in the Nickerie/Wageningen area are given here: January (173 mm), February (110 mm), March (114 mm), April (159 mm), May (242 mm), June (304 mm), July (240 mm), August (147 mm), September (59 mm), October (48 mm), November (78 mm), December (164 mm).

Long rainless periods are as a rule exceptional and even in the dry seasons (September-November, February-April) showers fall at irregular intervals. From this it follows that the duration of the diapause in *R. albinella* in the field may be rather short. As is shown by the results of the first series of the showering experiment, which by its start at December 1st was performed under conditions which correspond best with those in the field, a stubble destruction within one month after harvest will prevent the appearance of moths.

RESUME

L'INFLUENCE DES PRÉCIPITATIONS SUR LA DURÉE DE LA DIAPAUSE CHEZ LA CHENILLE MINEUSE DU RIZ RUPELA ALBINELLA *(CR.) EN SURINAM (AMÉRIQUE DU SUD).*

L'auteur a étudié la diapause chez le borer du riz *Rupela albinella* afin de trouver une base solide pour un programme de destruction des chaumes du riz. Les chenilles en diapause restent abritées dans le chaume après la récolte. L'influence des précipitations sur la durée de la diapause a été analysée expérimentalement. L' auteur observa une corrélation directe entre la quantité des précipitations et l'apparition des papillons. Ce „réveil" des chenilles en diapause est acceléré en fonction de la durée de leur séjour dans le chaume sec.

L'auteur suggère que la destruction des chaumes dans le mois suivant la récolte peut empêcher l'apparition des papillons.

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TERMINATION OF DIAPAUSE AND CONTINUOUS LABORATORY REARING OF THE SWEET CLOVER WEEVIL, *SITONA CYLINDRICOLLIS* FAHR.

BY

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Young adults of *Sitona cylindricollis* were collected in the field and treated in the laboratory with various temperatures and photoperiods to terminate the diapause.

Different methods of rearing weevils from the egg stage to mature adults are described and discussed.

The sweet clover weevil, *Sitona cylindricollis* Fahr. is well known as a pest of sweet clover in North America (BIRD, 1947, 1949; WILSON, 1951). Thorough studies on the biology and behaviour of the weevil and especially breeding of weevil-resistant varieties of sweet clover are desirable.

S. cylindricollis is a univoltine species with an imaginal diapause which starts in late summer. Diapause is indicated by an arrest of the development of the reproductive organs. The oviposition of the weevils in Manitoba does not start until the following spring with the onset of favourable weather conditions.

This restricts investigations on *S. cylindricollis* to the few summer months when the weevils are active in the field. A great number of experiments have to be compressed into this short time and very often they fail because even at this time the weevils may be scarce in some years or cannot be collected in the field because of adverse weather conditions.

Therefore mass rearings in the laboratory would be desirable, especially if several generations a year could be reared. Laboratory culture could provide homogeneous material and young and mature adults simultaneously throughout the year. In this endeavour the greatest difficulty is to overcome diapause in the young weevil. The following experiments were encouraged by observations on the pea leaf weevil, *S. lineatus* L. (HANS, 1959), a close relative of the sweet clover weevil, and by the paper of DAVEY (1956) on the physiology of the diapause in *S. cylindricollis*. In *S. lineatus*, oviposition in the laboratory started in October when the weevils were kept at 22° C and a 16 hour photoperiod and were fed with young pea leaves. DAVEY found that the oxygen uptake from August until September decreased in *S. cylindricollis* but increased slowly after October although the weevils were still kept at low temperatures. This suggests that diapause in the weevil had already begun to wane at that time.

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This paper describes the influence of different photoperiods and low temperature treatments on the termination of diapause in *S. cylindricollis.*

In the laboratory mature adults were reared from the egg stage.

MATERIAL AND METHODS

The insects used in the following experiments were collected in large numbers at the end of August 1959 in a second-year sweet clover field which was already harvested. A few weevils were gathered from their hibernating quarters in a second year sweet clover field in the middle of April 1960 as soon as the snow had melted.

In the laboratory the insects were treated as follows:

1. One group of 25 pairs was kept in the laboratory at a temperature of 25° C under fluorescent light at a 16 hour photoperiod of 300 ft-c intensity.
2. Another group of 25 pairs was treated exactly the same except that the photoperiod was 8½ hours.

In both groups each pair was placed in a separate plastic dish of 7 cm diameter and 1.5 cm height, with a hole in the lid covered with screening. Filter paper in the bottom of the dishes was moistened slightly every second day to keep the food plants fresh. On alternating days the weevils were fed young sweet clover leaves and the dishes were examined for eggs. Each group of dishes was placed in a large metal tray covered with Saran plastic film which helped to reduce drying of the leaves.

The remaining weevils were placed in plastic boxes in a dark room at 4 to 5° C. Once a week the containers were filled with fresh sweet clover foliage because the weevils were always feeding a little even at these low temperatures. At the same time the foliage provided a satisfactory humidity in the boxes. If the weevils were kept too moist they were killed very quickly by the fungus *Beauveria bassiana* (Bals.) (BIRD, 1949). Until February, weevils were taken from the cold room at intervals of nearly three weeks and maintained under the conditions described above.

The weevils collected in April from the hibernating quarters also were held at 25° C with 16 hour or 8½ hour photoperiods. This experiment was done to find out whether there was a difference in time of commencement of oviposition between weevils which spent the winter under natural conditions in the field and those which were kept in the cold room under artificial conditions. The number of days to oviposition was recorded. A female was regarded as mature when the first eggs, fertile or infertile, were laid. Since no gonad investigations were done, the exact date of male maturation was not established. The only indication of male maturation was mating of the pairs and fertile eggs laid by the female. This did not give any knowledge of the males which may have become mature earlier than the females. On the other hand it did not occur very often that the females laid infertile eggs for long, so that it can be assumed that both sexes mature at nearly the same time. Therefore, in the following results only the dates when oviposition started are given and this time is called pre-oviposition period.

To rear large numbers of insects most efficiently, sweet clover was planted in ordinary flats in the greenhouse. Since it was known for *S. lineatus* (ANDERSEN, 1931) and suggested to be the same for *S. cylindricollis* that the young weevil larvae feed mainly on the nitrogen nodules of the Leguminosae, the seeds of sweet clover were inoculated with nitrogen fixing bacteria1) before seeding. After six to eight weeks the roots of the plants were very well developed and showed many nitrogen nodules so that there was plenty of food for the young hatching larvae of *S. cylindricollis.*

The eggs of *S. cylindricollis* were lightly covered with soil on the surface of the flats and kept moist for eight to ten days until the eggs had hatched and the larvae reached the roots of the plants. Since adult weevils can be expected in six to eight weeks, depending on the ambient temperature, screened cages were put over the flats after six weeks to prevent the weevils from escaping. The young weevils all emerged at nearly the same time.

In another experiment mature egg laying weevils were put directly under screened cages on the sweet clover flats and exposed to 16 hours of light daily The temperature in the greenhouse varied very much during the season, averaging 24° C, ranging from 13 to 40° C. The young weevils emerged over a period of nearly two months and it was not possible to record dates of emergence so that age of individuals could be determined.

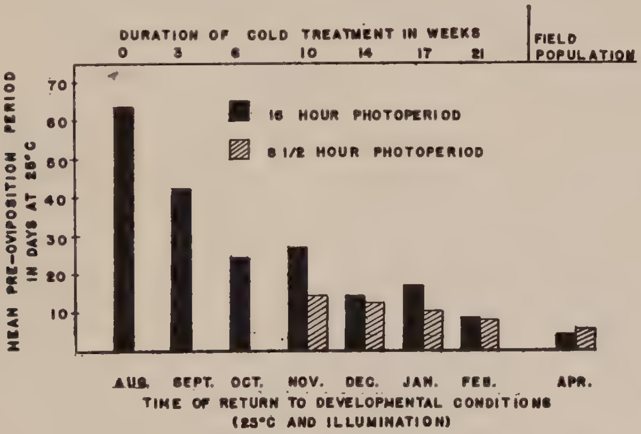

Figure 1. Effect of cold treatment and photoperiod on termination of diapause in *S. cylindricollis.*

RESULTS

In Figure 1, the effectiveness of the different treatments on the termination of diapause in *S. cylindricollis* is shown. Oviposition started without cold treatment in a 16 hour photoperiod at 25° C after an average of 63.8 days. In an 8½ hour photoperiod at 25° C no oviposition was observed.

If weevils were kept in a 16 hour photoperiod at 25° C after a three week cold treatment, they started to lay eggs in a shorter time, i.e. 42.2 days average after

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1) "Nitro Nox", Seed Inoculation for Clovers and Alfalfa. Steel Briggs Seed Ltd., Winnipeg, Manitoba.

return to developmental conditions. However the total duration of the experimental treatment remained nearly the same. At an 8½ hour photoperiod the weevils did not lay any eggs. A previous cold treatment of six weeks still more effective for hastening reproduction in weevils maintained at a 16 hour photoperiod at 25° C. Eggs were found after 24 days on the average. There was still no oviposition with weevils allowed ondy 8½ hour photoperiod. Again, the period required for the entire treatment remained approximately the same. A further prolongation of exposure in the cold room up to February progressively shortened the time until oviposition started under 16 and 8½ hour photoperiods at 25° C but increased the time required for the total treatment. Adults which were collected from the hibernating places in April responded to developmental conditions in the same way as weevils which were kept at low temperatures until February.

Insects kept at low temperatures until November (10 weeks) and longer started to lay eggs with either photoperiod. There was no significant difference in the time when oviposition started for the 16 and 8½ hour photoperiod. The percentage of mature females averaged 52% with the 16 hour photoperiod and 24% with the 8½ hour photoperiod following cold treatment. The difference was significant ($p < 0.05$). An analysis of variance showed that there was a significant difference among mean durations of pre-oviposition period at developmental conditions. There was a significant negative regression of the average pre-oviposition period on the time spent in the cold room ($p < 0.001$).

DISCUSSION

The results suggest that diapause in the weevils is ended around October. After that development of the gonads starts even at low temperatures but is accelerated by high temperaures and young leaves as food. The observations of DAVEY (1956) agree with this conclusion. The oxygen-uptake of weevils which were kept in the cold room during the entire winter decreased until the middle of October but increased slowly after that. The fat content of the weevils stayed more or less constant until December and decreased very suddenly after that time. Both facts indicate a higher rate of metabolism and the beginning of gonadal growth. EDELMAN (1951) had obtained similar results with the tenebrionid *Anatolica eremita.* Their oxygen-uptake decreased until January and increased after that, but the hibernation of the beetles under natural conditions lasted until April. In this case LEES (1955) suggested that the rising curve of oxygen consumption probably indicated the termination of diapause.

In none of the experiments conducted did all females oviposit. Always at least 30% of the adults remained in diapause or died very quickly. Perhaps optimal conditions for rearing them have not yet been realized. DE WILDE, DUINTJER & MOOK (1959) had similar experiences when they found that 20—30 per cent of Colorado potato beetles, *Leptinotarsa decemlineata* (Say) persisted in entering diapause when maintained under a long-day photoperiod.

The fact that diapause can also be terminated without cold treatment by a 16 hour photoperiod at 25° C indicates that a "long day" and low temperatures can be interchangeable in effect on the termination of diapause. In the latitude of Winnipeg the temperature is still high enough in August and plenty of young sweet clover is available, but imaginal diapause in the weevil will start, even when they are still active and feeding. Therefore it is assumed that diapause is induced by the shorter photoperiod (14 to 13 hours in August to September) in the young weevils. Experiments on the influence of the photoperiod on young adults which were kept at a 16 hour photoperiod right after emerging from the soil are not conclusive, but indicate that diapause can be completely prevented. From 20 pairs collected in the field in the end of July, four started to lay eggs after 18 days on the average. This indicates that the weevils start oviposition after a short period of feeding, as can occur in *L. decemlineata* (DE WILDE, DUINTJER & MOOK, 1959). One would expect a second generation in latitudes with a longer season (as in *S. lineatus* in Italy (HANS, 1959; ZANGHERI, 1952). A partial second generation has been observed in the latitude of Nebraska, U.S.A. (C. R. MANGLITZ, *pers. communication*).

If the weevils were collected in the field and the data of emergence not known, the most efficient way for rearing the sweet clover weevil in the laboratory was to keep the weevils at 5° C for six weeks and after that in a 16 hour photoperiod at 25° C. This is preferable to immediate exposure to warm, long day conditions because it conserves labour and greenhouse space although the time required is equal in both methods. It was found best to rear larvae separately from the adults. Cultures of adult weevils were kept in plastic dishes and fed on cut sweet clover leaves. From these cultures, eggs could easily be collected. Eggs were raked slightly into the soil of sweet clover flats. The soil was kept moist for ten days to protect the eggs and young larvae from desiccation. Since a predetermined number of eggs could be introduced in this way all at one time, a basis was provided for estimating survival rate as well as the age of the emerging weevils.

If mature weevils were placed in cages on the sweet clover flats and allowed to oviposit *ad libitum*, the following disadvantage was found: the soil could not be kept suitably moist for maximal survival of the larvae. The high optimal humidity for the larvae was detrimental to the adults because the fungus *Beauveria* thrives under moist conditions. Also, the adults ingested less food at high humidities and laid fewer eggs, as was observed also with *S. lineatus* (HANS, 1959). By the method used here a culture of *S. cylindricollis* was reared through two generations in eight months. Three generations a year should be possible.

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ZUSAMMENFASSUNG

BEENDIGUNG DER DIAPAUSE UND KONTINUIERLICHE LABORATORIUMSZUCHT DES SÜSSKLEEKÄFERS, SITONA CYLINDRICOLLIS FAHR.

Jungkäfer von *Sitona cylindricollis* wurden im Herbst in Süsskleefeldern gesammelt und im Laboratorium zur Eiablage gebracht. Die Diapause wurde nach ca. 9 Wochen durch eine

16-stündige Photoperiode, 25° C Umgebungstemperatur und junge Süsskleelaub als Nahrung beendet. Eine Kältebehandlung in 5° C für 3 oder 6 Wochen und nachfolgender Aufenthalt im Langtag bei 25° C waren ebenfalls wirksam und lösten nach einer Gesamtzeit von 9 bis 10 Wochen Eiablage aus. In diesen Grenzen konnte eine vorhergehende Kältebehandlung eine ebenso lange Zeit in entwicklungsfördernden Bedingungen ersetzen ohne die Gesamtzeit, die bis zum Beginn der Eiablage benötigt wurde, zu verlängern.

Eine weitere Verlängerung der Kältebehandlungen verlängerte nur die Gesamtdauer der Behandlungszeit, obwohl die Zeit, die nach Rückkehr in entwicklungsfördernde Bedingungen bis zur Eiablage notwendig war, verkürzt wurde. Andrerseits machten diese längeren Kältebehandlungen eine Langtags-Photoperiode überflüssig.

Zwei Generationen von *S. cylindricollis* wurden durchgehend im Gewächshaus gezüchtet. Es sollte möglich sein, 3 Generationen Käfer pro Jahr im Laboratorium zu züchten. Die Zuchtmethoden werden beschrieben und diskutiert.

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STIMULATING EFFECT OF DDT TREATMENT OF COTTON ON WHITE FLIES *(BEMISIA TABACI* GENN.; ALEURODIDAE) IN THE SUDAN GEZIRA

BY

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Treatments of cotton with DDT, Toxaphene, Delnav[1]) and Endrin gave a reduction of the number of adults and larvae of *Bemisia tabaci* during 2—3 weeks after treatment. Compared to untreated, Toxaphene, Delnav and Endrin gave a further reduction of larvae during the following weeks, whereas DDT caused resurgence of larvae at 14 days after treatment and of adults at 20 days after treatment.

Several possible explanations for this phenomenon are considered. During the time of spraying, the generation which caused the resurgence was in the egg stage. The eggs of Aleurodidae are in physiological contact with the plant on which they occur.

Physiological changes in the plant, favourable to the insects, causing a decreasing natural mortality of the insect population during the egg stage, may account for this stimulative effect which is exclusive for DDT or its formulation. The other possible explanations are considered to be much less probable.

It has been known for some time that DDT treatments influence the growth of a number of plant species in the absence of insect pests. CHAPMAN & ALLEN (1948) treated various crops with DDT and found growth stimulation of beans, tomatoes, potatoes and other vegetables at very low concentrations, which growth stimulation was caused both by DDT spray and dust. At higher concentrations injuries were found. ALLEN & CASSIDA (1951) developed a method to test the effect of toxicants on the growth of isolated bean seedlings. With this standard method it could be proved that DDT stimulated plant growth in an unbuffered nutrient solution in which no auxin was present, and inhibited growth when an auxin was present. This indicates a relationship between the action of DDT and that of auxins in plant metabolism.

In field experiments at Tokar in Sudan GOODMAN (1955) treated cotton plants of the variety X-1730-A fortnightly with 0.2% DDT and found a higher number of flowers on three representative dates in the sprayed plots than in the unsprayed plots. Differences were found also in plant heights, number of green bolls and other properties. Insect infestation was very low at the time. GOODMAN concluded that direct growth stimulation by DDT or its formulation had occurred.

JOYCE (1955a) found a marked enlarging effect on leaf size, caused by DDT spraying in field experiments in Sudan Gezira, even if insects had been killed by Parathion. The same author (JOYCE, 1958) found that there was a correlation between the population size of White flies (*Bemisia tabaci* Genn.) and the nitrogen content of the leaves.

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[1]) Active ingredient: 2,3-*p*-dioxanedithiol S,S-bis (0,0-diethyl phosphorodithioate).

White fly is a serious pest of cotton in the Sudan, especially in the South Gezira where DDT has been sprayed annually over a period of many years.

Joyce (1955b, 1959) mentioned that DDT sprays, after an initial, but ineffective reduction gave rise to an increased rate of breeding of the White flies. Even the decline of the population in the later part of the season, which is a normal phenomenon, appeared to be delayed by four to six weeks by DDT spray. Joyce & Roberts (1959) showed that the DDT treated plots were significantly more highly infested than the untreated plots from 26 to 70 days after spraying, though no difference appeared during the first 17 days after spraying.

My experiences with the stimulating effect of DDT on the White flies, gained during a stay of some months at the Gezira Research Station in the course of a field experiment, lead to the supposition that a relation exists between the growth stimulation of the cotton plants as found by Goodman and Joyce, and the stimulation of White fly population, as mentioned by Joyce, both occurring after treatment with low DDT concentrations.

METHODS

In a field experiment with the cotton variety X-1730-A one treatment was given with DDT, Toxaphene, Delnav and Endrin in five replicates of 0.25 acres subplots. The details are given elsewhere (van der Laan 1961). The chemicals were applied as a low volume spray, the DDT treatment at a dosage of 0.5 lb active ingredient per acre. Two days before treatment and 5, 9, 14, 19 and 25 days after treatment the number of living third and fourth instar larvae were counted in the laboratory under the microscope (enlargement 16 ×) on 100 leaves per subplot. During the same period, independently of these counts, the number of adult White flies was counted also. These counts were carried out early in the morning in the field by inspecting the lower surface of 120 leaves per subplot.

RESULTS

White fly larvae. As can be seen from fig. 1 (untreated subplots) the White

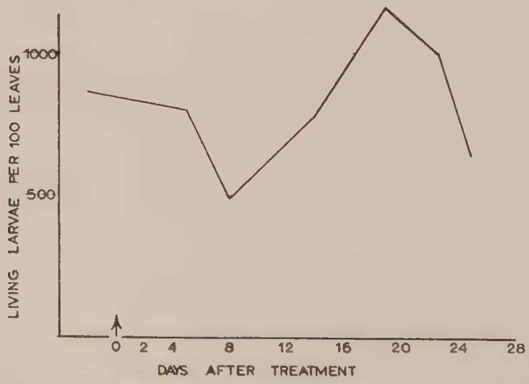

Fig. 1. Course of White fly population during the experiment, as counted in the untreated plots.

fly population level fluctuated during the time of observation with a trend to decrease at the end. This is normal at that time of the season.

All treatments gave a decrease of *Bemisia* larvae 5 and 9 days after treatment. The next counts which were carried out 14 and 19 days after treatment showed a further relative reduction in numbers as compared with the untreated

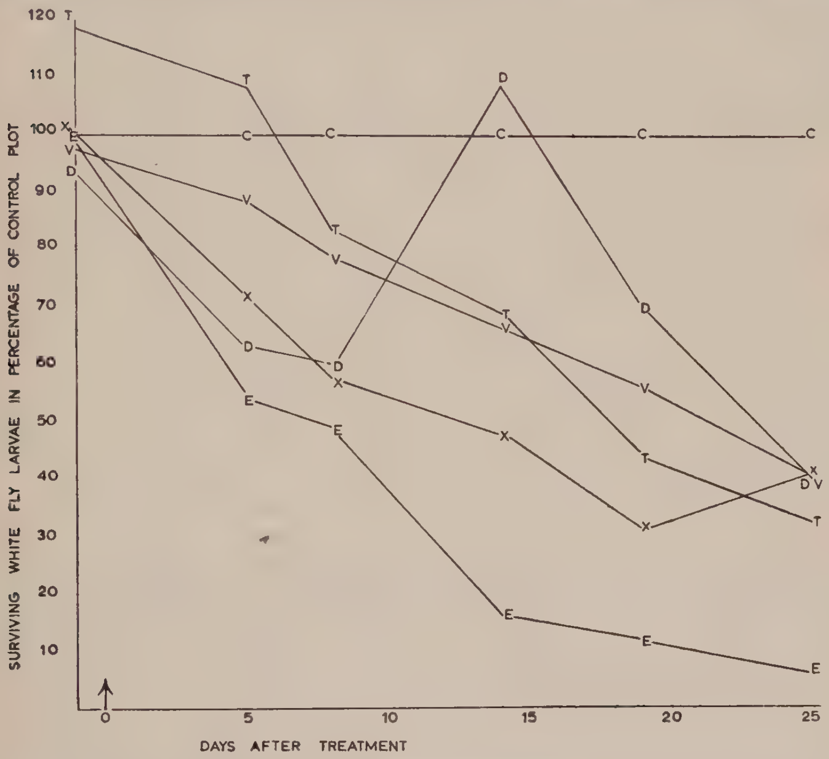

Fig. 2. Effect of treatment of different insecticides against 3rd and 4th instar White fly larvae. Untreated plots = 100; D = DDT; T= Toxaphene; E = Endrin; V = Delnav; X = Delnav-X.

TABLE I

Number of living 3rd and 4th instar larvae of White fly per plot (100 leaves)

	Replicate	DDT	Toxaphene	Delnav-X	Delnav	Endrin	Check
One day before treatment	1	97	220	88	113	153	131
	2	141	142	151	144	95	146
	3	218	244	221	219	228	272
	4	150	291	288	144	234	168
	5	202	120	126	219	156	142
	Totals	808	1017	874	839	866	859
14 days after treatment	1	281	90	86	189	14	227
	2	238	61	64	127	26	175
	3	56	98	58	72	31	172
	4	172	168	117	108	17	83
	5	105	117	29	25	36	126
	Totals	852	534	374	521	124	783

control plots except for the DDT plots which showed a surprising increase in the numbers of 3d and 4th instar larvae (Fig. 2; Table I).

For analysis of variance, the counts obtained from individual replicates were transformed logarithmically. On the fourteenth day after treatment the difference between the larval population in the DDT plot and the other plots was significant at the 0.05 level. The figures of next counts show that the DDT peak had vanished.

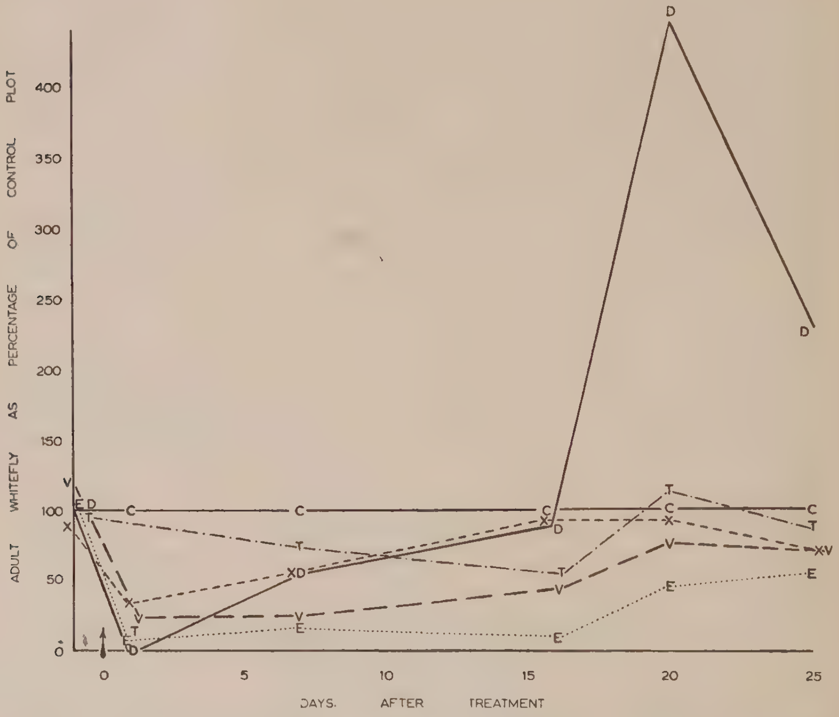

Fig. 3. Effect of treatment of different insecticides against White fly adults. Untreated plots = 100; D = DDT; T = Toxaphene; E = Endrin; V = Delnav; X = Delnav-X.

White fly adults. Similar results were found when counting the adults of the White flies in the same experiment (Fig. 3, Table II). Here the DDT peak comes later, namely 20—25 days after treatment and the difference is highly significant between this DDT treated plot and the other plots ($P < 0.01$). The time interval between the peaks of larval adult counts is explained by the time needed for development from the third and fourth larval stage to the adult stage.

DISCUSSION

If we try to find an explanation for the stimulative effect of DDT on the White fly population, several causes may be taken into consideration.

1. Joyce (1955b) supposes that DDT kills the natural enemies of the White fly, for instance the larval Hymenopterous parasite *Eretmocerus diversiciliatus*

TABLE II

Number of adult White flies per plot (120 leaves)

	Replicate	DDT	Toxaphene	Delnav-X	Delnav	Endrin	Check
One day before treatment	1	36	42	21	31	46	42
	2	41	24	30	41	50	37
	3	26	23	41	31	28	32
	4	40	42	62	34	29	30
	5	29	29	23	40	19	29
	Totals	172	160	157	177	172	170
21 days after treatment	1	61	16	29	17	20	30
	2	62	44	42	30	14	39
	3	222	34	25	10	16	33
	4	323	55	35	14	13	32
	5	57	43	19	58	11	26
	Totals	725	192	150	129	74	160
26 days after treatment	1	25	13	17	8	16	19
	2	8	16	24	23	13	32
	3	113	15	13	13	15	11
	4	49	30	11	10	4	19
	5	41	17	6	17	6	20
	Totals	236	91	71	71	54	101

Silv. (Aphelinidae) which occurred also rather frequently in my experiment. It is found (Norman & La Croix, Ann. Rep. Gezira Research Station 1954/1955) that significant and important declines in the number of parasitised pupae followed DDT spraying. Part of the resurgence effect may be ascribed to this cause. However, the other insecticides used in the experiment were just as toxic, or even more toxic to the parasites than DDT, and caused no increase of White fly.

2. Another possible explanation of the effect is given by Joyce (1955b). The DDT treatment would have destroyed the population of Jassids (*Empoasca lybica* De Berg), and lack of competition would be the cause of the rise of the White flies. In the experiments of Joyce & Roberts (1959), the only insecticide used was DDT. In my experiments, however, other insecticides were included as well; they all were effective against the Jassids (van der Laan 1961) and no peak in number of White flies occurred in the plots treated with either Toxaphene, Delnav or Endrin.
3. It is found that DDT at low concentrations stimulates egg production of the Fruit tree Red Spider (*Metatetranychus ulmi* Koch) (Hueck, Kuenen, den Boer & Jaeger-Draafsel 1952), and also of the grain weevil *Sitophilus granarius* (L.) (Kuenen 1958).

In my experiment the adult population was reduced to a very great extent right after the treatment, in the DDT treated as well as in the other treated plots. Also during a period of about one week after treatment the adult population is still very low. Taking into consideration that the life cycle of the White fly is 14 days or longer, it is hard to understand, if not impossible, that the resurgence is caused by increased egg laying, because this increased egg laying would have taken place in the period of reduced White fly population (1—7 days after application).

As none of these possibilities is applicable to the present case, some other explanation must be sought. In the absence of any proof, such explanation must remain purely conjectural, pending further studies.

As is the case with many insect populations in the tropics, all stages of *Bemisia tabaci* are present simultaneously. From the figures the conclusion can be made that the generation which caused the peaks after DDT treatment, was apparently in the egg stage at the time of treatment. During the egg stage contact between plant and eggs of Aleurodidae exists; the eggs can take up water through their stalks from the plant (WEBER, 1931), and probably substances dissolved in water also.

On the other side, it is known that the growth of cotton plants can be stimulated by DDT at low concentrations (GOODMAN, 1955; JOYCE, 1955a).

Now a hypothesis is formed that changes in the composition of the food are inducing some change in the rate of viability of the eggs. Normally the egg stage of white fly lasts about 7 days, and natural mortality is very high. The influence of the DDT treatment is supposed to result in a decrease of the natural mortality of the eggs, and hence a peak in the population occurs 2—3 weeks later. Before the peak comes up, a slight decrease of the population is demonstrated, due to the lethal effect of the treatment on the larvae. Notwithstanding the fact that part of the larval population will be killed by the residue of DDT, a temporary increase of the White fly population results.

If the stimulative effect of DDT on *Bemisia* is caused by interaction of changes in the plant and the natural mortality of the insect population, local differences, even between the leaves of one cotton plant, may influence this effect. The large differences in numbers of White flies between the separate subplots (Table I & II) treated with DDT, may be caused in that way. It is not surprising that the effect is most prominent, if — as was the case in my experiment — the natural population of *Bemisia tabaci* was no longer increasing (Fig. 1).

JOYCE & ROBERTS (1959, fig. 10) found a resurgence which lasted longer than the one which was found in this experiment. Their experiment contained very large plots. It may be supposed that in my experiment the resurgence faded out more quickly because of the influence of adjacent plots.

Many thanks are due to Dr. A. D. Hanna, Director and Ir. L. Razoux Schultz, Entomologist of the Gezira Research Station for their continuous help and kind hospitality, to Mr. Abdel Rachman Agabani for valuable assistance and last but not least to Ir. A. Overeem and Ir. K. de Vries of Hercules Powder Company, The Hague, Netherlands for their noteworthy cooperation. The statistical analysis of the results was taken over by the "Centrum voor Landbouwwiskunde" at Wageningen, and my sincere thanks are due to Mr. M. Keuls for his help.

ZUSAMMENFASSUNG

*DIE STIMULIERENDE WIRKUNG DER DDT-BEHANDLUNG VON BAUMWOLLE AUF DIE MOTTENSCHILDLAUS (*BEMISIA TABACI *GENN., ALEURODIDAE) IM SUDAN GEZIRA*

Der Erfolg einer Prüfung von Insektiziden gegen die Aleurodide *Bemisia tabaci* auf Baumwolle im Sudan Gezira wird mitgeteilt. Die Mittel DDT, Toxaphen, Delnav und Endrin ergaben anfangs eine Abnahme der Larval- und Imaginalpopulationen während zwei bis drei Wochen nach der Behandlung. In den nachfolgenden Wochen setzte sich diese Abnahme auf den mit Toxaphen, Delnav und Endrin behandelten Flächen fort. In den mit DDT gespritzten Beständen nahm die Population der Larven jedoch schon zwei Wochen nach der Behandlung schnell zu und eine Woche später hatten sich auf diesen Flächen auch die Imagines zahlenmäßig stark vermehrt.

Nach einer Erklärung dieser Stimulation durch DDT wird gesucht. Es ist nicht anzunehmen, daß die Ursache in der Abtötung von Parasiten oder konkurrierenden Insekten zu suchen ist, da die anderen Insektizide diese Wirkung ebenso gut wie DDT entfalten, aber keine Stimulation verursachen.

Frühere Untersuchungen haben Beweise dafür erbracht, daß geringe Konzentrationen von DDT den Wuchs der Baumwollpflanzen fördern, ohne daß Insekten im Spiele sind. Weiter hat seinerzeit WEBER (1931) gefunden, daß die Eier der Aleurodiden etwas Flüssigkeit aus den Blättern aufnehmen können. Da sich die Generation, welche die Stimulation erfuhr, während der Spritzungen gerade im Eistadium befand, wird die Hypothese aufgestellt, daß eine physiologische Einwirkung des DDT auf die Pflanzen die — normalerweise — hohe Mortalität der Aleurodiden-Eier so sehr vermindert, daß eine Zunahme der *Bemisia*-Population erfolgt.

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STUDY ON THE FREE AMINO ACID COMPOSITION OF MID-INTESTINE IN SEVERAL SPECIES OF LAMELLICORN BEETLES

BY

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The patterns of the free amino acid composition in the mid-intestine tissue of twelve species of Lamellicornia (Coleoptera) were compared by means of paper chromatography. As tested in *Allomyrina dichotomus* L., the contents of amino acids of the tissue seemed to be constant not only among individuals of the same sex, but also among those of both sexes in a single species. The beetles tested could be divided into five groups by their amino acid constituents of the mid-gut. This division does not necessarily parallel the taxonomic classification, nor the anatomical grouping of the digestive system. The food habits of the beetles seem to have a certain bearing upon the amino acid composition of their mid-intestinal tissue.

Since the simple method of chemical analysis by paper chromatography was developed, intensive studies have been carried out on the free amino acid constituents in insect tissues, and a considerable amount of information has been accumulated on this subject. From the view-point of taxonomists, this is probably a fascinating new approach to the species problem or phylogenetic relations. Different species of insects, and in certain cases even different strains of the same species, differ in the amino acid composition, as demonstrated by many workers in mosquitoes belonging to *Culex, Aedes*, and *Anopheles* (BALL, 1952, 1953; LEWALLEN, 1957; MICKS & ELLIS, 1951, 1952; MICKS, 1954, 1956; MICKS & GIBSON, 1957) and in pomace-flies, *Drosophila* spp. (BLUMEL & KIRBY, 1948).

The present work deals with the results of a comparative study on the content of free amino acids in the mid-gut tissue of twelve species of the Lamellicornia, which is considered as a distinct natural group. The aim is to correlate these results with those of anatomical studies of this particular organ. Based on the structure of the mid-intestine, the phylogenetic relations among families or subfamilies in the Lamellicornia have been discussed elsewhere (UMEYA, 1960), and it will be interesting to know to what extent the systematic relations seen in the structure and function of the digestive system are connected with the pattern of the free amino acid composition.

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MATERIAL AND METHOD

The samples of mid-gut for the paper chromatographic analysis were obtained from the following species of six families[1]).

Family Lucanidae
  Eurytrachellelus titanus Boisd. (2 ♂♂ 2 ♀♀, Tokyo, August 1958)
  Psalidoremus inclinatus Motsch. (3 ♂♂ 1 ♀, Tokyo, August 1958)
Family Cetoniidae
  Torynorrhina japonica Hope (5 ♂♂ 2 ♀♀, Tokyo, August 1958)
Family Melolonthidae
  Allomyrina dichotomus L. (16 ♂♂ 11 ♀♀, Tokyo, August 1958)
  Melolontha japonica Burm. (5 ♂♂ 2 ♀♀, Tokyo, August 1958)
  Anomala rufocuprea Motsch. (6 ♂♂ 3 ♀♀, Tokyo, August 1958)
  Anomala cuprea Hope (3 ♂♂ 3 ♀♀, Tokyo, August 1958)
Family Scarabaeidae
  Onthophagus lenzii Har. (12 ♂♂ 5 ♀♀, Tokyo, August 1958)
  Copris pecuarius Motsch. (3 ♂♂ 3 ♀♀, Nagano, August 1958)
  Copris ochus Motsch. (3 ♀♀, Nagano, August 1958)
Family Geotrupidae
  Geotrupes laevistriatus Motsch. (8 ♂♂ 9 ♀♀, Hokkaido, October 1958)
Family Passalidae
  Cylindrocaulus patalis Lewis (9 ♂♂ 8 ♀♀, Shikoku, March 1958; February 1959)

The mid-intestines were taken from living specimens of adult beetles, and the debris of muscles, tracheae and Malpighian tubules attached on the gut were removed. The tracts were cut open longitudinally in water and ingested food materials were thoroughly taken off by washing with water. The tissues were then ground in a mortar with 70 per cent aqueous alcohol and filtered. Filtrate was concentrated and preserved in a refrigerator until analysis.

The two dimensional paper chromatographic analysis was carried out at a temperature of 25° C, using 80 per cent aqueous solution of phenol as the first solvent and a mixture of butanol, acetic and water (4 : 1 : 2) as the second solvent on a filter paper sheet of 30 × 30 cm in size (Tôyô Rosi Co., No. 50). After running, ninhydrin solution in butanol was sprayed on for colour development.

In each species the tissues from several individuals were used together for a single analysis. With these procedures the analysis gave consistent results, and the amino acids detected on the paper sheet were readily identified. A precise quantitative analysis, however, could not be made, because neither the amount nor the concentration of the samples could be kept constant throughout the experiments. In the following accounts, therefore, the relative abundance of each amino acid is arbitrarily judged from the size and/or colour-intensity of the developed spots, and expressed roughly as small (+), moderate (+++), large (+++++), etc. The present purpose could be attained by this approximate comparison.

RESULTS AND DISCUSSION

Individual variations in Allomyrina dichotomus

Before extensive analyses, some tests were made in order to determine the in-

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[1]) The arrangement of the families listed here follows the tentative system introduced by UMEYA (1960).

TABLE I

Free amino acid composition in mid-gut tissue of Allomyrina dichotomus *showing individual varia*

Arabic numerals indicate individual number

Sample / Amino acid	males				females
	1	2	3	4	1
Aspartic acid	++	+	+	+	++
Glutamic acid	+++	+++	+++	+++	++++
Cystine	+	+	+	+	+
Glycine	+++	++++	+++	++++	+++
Serine	+	++	++	++	+
Alanine	++++	++++	++++	++++	++++
Arginine and/or Histidine	++++	+++++	+++++	+++++	+++++
Tryptophane	+	++	+	++	++
Proline	+++++	+++++	++++	+++++	+++++
Tyrosine	++	+	+	+	++
Valine	++++	++	+++	+++	+++
Leucine and/or Iso-leucine	++++	+++	++++	++++	+++++
Lysine	++	++	++	++	++

dividual variations within a species in both the amount and kind of free amino acids, and also to detect the sexual difference. For this purpose the mid-gut of *Allomyrina dichotomus* was used, because it was so large in size among the Lamellicornia available that the analysis of its amino acid constituents was easily made with a sample taken from one individual. Samples were prepared from four male beetles and four female beetles immediately after they were captured in the field, and from four males starved for a week in the laboratory. The results are set out in Table I.

The table shows that fifteen amino acids were detected from the mid-gut of most individuals tested, except from two starved ones in which the presence of cystine was obscure. In all cases where cystine was detected, it occurred only in a very small amount, and the failure of detection in the two starved beetles was probably due to the technical defect rather than to the absence of this acid. Relative amounts of each amino acid varied to a certain extent among individuals, but there was no remarkable fluctuation. This may imply that the amino acid composition is fairly stable within a species.

Comparison of amino acid composition among species

The results of the analyses of free amino acids from the mid-gut of the abovementioned twelve species are given in Table II. In these analyses, eighteen amino acids were detected; among these, proline, valine and eleven other acids were found in every species tested. Cystine and tryptophane were not found in certain species; but their absence was not certain, since even the other species have only a trace of these acids. Generally speaking, the amino acid composition of the intestine of these beetles is closely similar qualitatively. Certain amino acids were,

females		males (fasting sample)				Mean
3	4	1	2	3	4	
++	++	+	+	+	+	+(+)
+++	+++	+++	+++	+++	+++	+++
+	+	+	+	+	+	+
+++	++	++++	+++	+++	+++	+++
+	++	++	++	++	++	++
+++	++++	+++	+++	++++	++++	++++
+++	++++	++++	++++	++++	+++++	++++(+)
++	++	++	++	++	++	++
+++	+++++	+++++	+++++	+++++	+++++	+++++
+	+	+	+	+	+	+
++	+++	+++	+++	+++	+++	+++
+++	++++	+++++	++++	++++	++++	++++
++	++	++	++	++	++	++

however, restricted within narrow limits: phenylalanine was thus detected only in the Scarabaeidae and the Passalidae, threonine in *Copris pecuarius*, and an undetermined amino acid (Rf. 7.8, 5.5) in the Cetoniidae.

On the other hand, the relative amount of amino acid varied considerably among species, and thus the beetles can be classified into the following five groups according to the relative abundance of the amino acids in their mid-gut.

Group I : *Eurytrachellelus titanus, Psalidoremus inclinatus* (Lucanidae);
Torynorrhina japonica (Cetoniidae);
Allomyrina dichotomus (Melolonthidae).
Group II : *Melolontha japonica, Anomala rufocuprea, Anomala cuprea* (Melolonthidae).
Group III : *Onthophagus lenzii, Copris pecuarius, Copris ochus* (Scarabaeidae).
Group IV : *Geotrupes laevistriatus* (Geotrupidae).
Group V : *Cylindrocaulus patalis* (Passalidae).

In the beetles of Group I the occurrence of tryptophane is very conspicuous as compared with the other beetles, and they are also characterized by having higher amounts of proline. Within this group, the four species tested showed a remarkable similarity in the amino acid composition except one cetoniid species which had an undetermined acid. The beetles of Group II had a larger amount of glycine than the others, and those of Group III had two times as much tyrosine as the rest. The beetles of Group III were also characterized by a high content of lysine and the presence of phenylalanine. Phenylalanine was also found in the Passalidae alone. Three species belonging to Group III showed similar composition of free amino acids in both quality and quantity, with the exception that *Copris pecuarius* had a smaller amount of threonine.

Group IV and Group V were represented by the Geotrupidae and Passalidae,

TABLE II

Free amino acid composition in mid-gut tissue of Lamellicorn species.

Family	Lucanidae		Cetoniidae	Melolonthidae	
Species	*Eur. titanus*	*Psal. inclinatus*	*Tor. japonica*	*All. dichotomus*	*Mel. ja*
Number of tests	5	3	5	18	
Aspartic acid	++	+(+)	++	+(+)	-
Glutamic acid	+++	+++	++++	+++	+-
Cystine	(+)	+(+)	++	+	
Glycine	++	++	+++	+++	++-
Serine	+	+	++	++	-
Alanine	++++	++++(+)	++++	++++	+-
Arginine and/or Histidine	++++	++++	++++	++++(+)	+-
Tryptophane	++	++	++	++	—
Proline	+++++	+++++	+++++	+++++	+-
Tyrosine	+	+	+	+	
Valine	++	+++(+)	++++	+++	
Phenylalanine	—	—	—	—	—
Leucine and/or Iso-leucine	+++(+)	++++	++++	++++	+-
Lysine	++	+(+)	++	++	
Thereonine	—	—	—	—	—
x 1 (unknown)	—	—	+++	—	—

respectively. The former were characterized by rather smaller amounts of arginine and/or histidine and lysine, and the latter by a large amount of phenylalanine.

Besides these major differences distinguishing the groups, there may be minor variations between different species within each group. On the other hand, there were certain common features in all the species. The contents of aspartic and glutamic acids, and leucine and/or iso-leucine were almost constant in different species. Furthermore, the beetles examined had invariably a large amount of proline in their mid-gut.

The digestive systems of the beetles used in the present study are illustrated in Figure 1. These were dwelt upon in detail in a previous paper (UMEYA, 1960). Present interest is directed to the relations between the amino acid constituents and the structural characteristics of the organ. Comparing these diagrams with the present data of chemical analyses, it is evident that Groups III, IV and V of beetles, as represented by their amino acid composition, correspond perfectly with the classification, according to the morphology of the intestine. In Group I, however, there are three different anatomical types of gut, namely the lucanid-, the cetonid-, and the melolonthid-types. *Allomyrina dichotomus* could be attributed to Group I for its amino acid composition despite its morphological features in common with the beetles belonging to Group II. In Group II, the morphological characteristics are in good accord with the chemical analyses.

These facts suggest that in the Lamellicornia the pattern of the amino acid composition of the mid-intestinal tissue is not necessarily correlated with the systematic or the phylogenetic position of the species. Although there is little connexion

Melolonthidae		Scarabaeidae			Geotrupidae	Passalidae
An. ocuprea	*An. cuprea*	*Onth. lenzii*	*Copris pecuarius*	*Copris oehus*	*Geotr. laevistriatus*	*Cyl. patalis*
5	6	6	7	5	8	10
++	++	+	++	++	+(+)	++
+++	++++	++++	++++	+++	++(+)	++++
++	++	++	+	—	—	(+)?
+++	+++++	+++	+++	++	++(+)	++
++	+(+)	++	++	+	++	+
+++	+++	++++	+++	+++	+++(+)	+++
+++	++++	++++	++++	++++(+)	++	+++
+	(+)?	—	+	(+)	(+)	+++++
+++	++++	+++++	+++	++++	+++	+++++
+	+	++(+)	++	++	+	+
+(+)	+++	++++	+++	+++(+)	+++	+++
—	—	+	+	+	—	+++++
+++	+++	++++	++++	++++	+++++	+++
++	++	++++	+++++	+++++	+	++
—	—	—	+	—	—	—
—	—	—	—	—	—	—

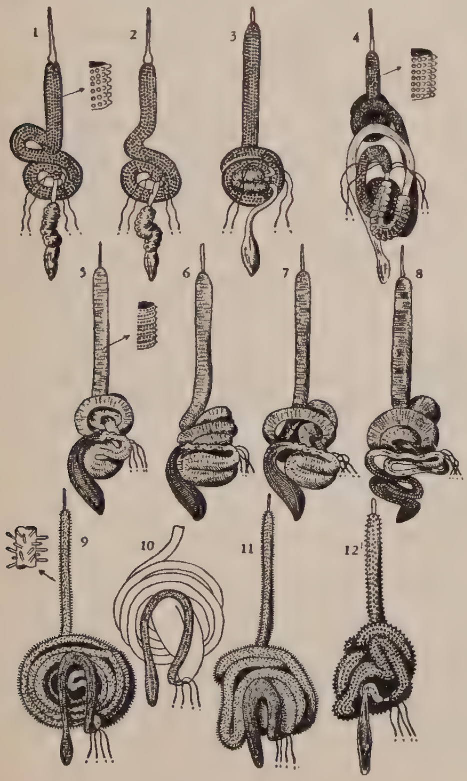

Figure 1. The digestive system of lamellicorn species tested:

1. *Psalidoremus inclinatus* (Lucanidae)
2. *Eurytrachellelus titanus* (Lucanidae)
3. *Torynorrhina japonica* (Cetoniidae)
4. *Cylindrocaulus patalis* (Passalidae)
5. *Anomala rufocuprea* (Melolonthidae)
6. *Anomala cuprea* (Melolonthidae)
7. *Melolontha japonica* (Melolonthidae)
8. *Allomyrina dichotomus* (Melolonthidae)
9. *Copris ochus* (Scarabaeidae)
10. *Copris pecuarius* (Scarabaeidae)
11. *Onthophagus lenzii* (Scarabaeidae)
12. *Geotrupes laevistriatus* (Geotrupidae)

between the anatomical structure of the digestive system and the food habit (UMEYA, 1960), the amino acid composition of the tissue has a certain bearing upon the food habits of the species, it seems (see Table III).

TABLE III

Food habit of the species.

Family	Species	Food habit
Lucanidae	*Psalidoremus inclinatus*	Succitrophic
	Eurytrachellelus titanus	Succitrophic
Cetoniidae	*Torynorrhina japonica*	Succitrophic
Melolonthidae	*Allomyrina dichotomus*	Succitrophic
	Anomala rufocuprea	Phyllophagous
	Anomala cuprea	Phyllophagous
	Melolontha japonica	Phyllophagous
Scarabaeidae	*Copris ochus*	Coprophagous
	Copris pecuarius	Coprophagous
	Onthophagus lenzii	Coprophagous
Geotrupidae	*Geotrupes laevistriatus*	Coprophagous Zoosaprophagous
Passalidae	*Cylindrocaulus patalis*	Necroxylophagous

The nectar-feeding species belong exclusively to Group I in the amino acid composition of their gut tissue, the leaf-eating ones to Group II, and most of the dung-feeders to Group III. The geotrupid beetle, which feeds on both dung and carrion, shows characteristics of Group IV in the amino acid composition of the gut, and the unique Passalid beetle, which feeds on decaying wood, also shows a peculiarity in the chemical traits of the gut and belongs to Group V. However, it should be pointed out that, although the scarabaeid and geotrupid beetles have similar food habits, their digestive systems differ considerably in structure as well as in the pattern of the amino acid composition. The results of morphological studies suggest that the phylogenetic background takes a more important part in moulding the anatomical features of the digestive system than the present-day food habits. In this context these results may offer interesting materials for inquiry.

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RESUME

RECHERCHE SUR LA COMPOSITION DES ACIDES AMINÉS LIBRES DANS L'INTESTIN MOYEN DE QUELQUES LAMELLICORNES ADULTES (COLÉOPTÈRES)

Ce travail apporte des données sur la composition des acides aminés libres dans le tissu de l'intestin moyen de 12 espèces des lamellicornes adultes. Les espèces étudiées sont divisible à 5 troupes par les types caractéristiques des acides aminés libres. Avec les relations morphologiques et alimentaires, certains aspects de cette classification sont mis en évidence dans les espèces étudiées.

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HOST PLANT PREFERENCE OF CEREAL APHIDS IN THE FIELD IN RELATION TO THE ECOLOGY OF BARLEY YELLOW DWARF VIRUS

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Observational data obtained by exposing *Avena sativa* L., *Phleum pratense* L., *Lolium perenne* L., *Poa pratensis* L., and *Zea mays* L. to the aerial aphid population indicated food plant preference as resulting in a greater number of settlers on the preferred host, on which migrants also remained longer and deposited more nymphs than on the less suitable hosts. This behavioural pattern of alate *Rhopalosiphum padi-fitchii* is discussed in relation to the epidemiology of barley yellow dwarf virus disease.

Field observations of aphids occurring on grain and grasses in Wisconsin, U.S.A., have shown that the two common species, *Macrosiphum avenae* (*granarium*) (Fabricius) and aphids of the *Rhopalosiphum padi-fitchii*[1]) complex were rarely encountered on the more common grasses (ORLOB & MEDLER, 1961). Records from other areas suggested, however, that cereal aphids may settle more frequently on grasses (OSWALD & HOUSTON, 1953; PATCH, 1938) but so far hardly anything is known of the extent and circumstances of infestation of grasses in nature.

Other workers have successfully reared cereal aphids on different grasses under greenhouse conditions (COON, 1959, c.f. ROCHOW, 1961). This does not necessarily prove a similar host plant acceptance in the field, which indeed may be distinctively different from an experimental initiation of populations in the greenhouse where aphids did not colonize plants of their own accord (ORLOB & MEDLER, 1961). Apart from behavioural patterns, host selection and preference assumes additional interest if the role of cereal aphids in virus transmission is considered.

Barley yellow dwarf virus, a widely distributed, persistent virus of Gramineae is harboured in various perennial grasses from where it may be acquired by parts of the vector population (c. f. ROCHOW, 1961).

A better understanding of virus epidemiology can only be gained if the available one-sided information on the relation between aphids and their hosts under greenhouse conditions is extended and inferred more frequently from field observations. In view of the importance grasses may have in the epidemiology of yellow dwarf, the host preference which aphids excercise among Gramineae in the field has been studied.

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[1]) Because of the close taxonomic similarity of *R. padi* (L.) and *R. fitchii* (Sanderson), the two species must be lumped together for all practical purpose. For further details see RICHARDS (1960).

MATERIAL AND METHODS

Three grasses of general nearctic distribution were selected, which also have been previously incriminated as carriers of barley yellow dwarf virus (c.f. ROCHOW, 1961). For comparison, oats (*Avena sativa* L., variety Clintland) was chosen as the preferred host of several cereal aphids, whereas corn (*Zea mays* L., variety Golden Bantam) served as a non-host plant. Timothy (*Phleum pratense* L.), perennial ryegrass (*Lolium perenne* L.), Kentucky bluegrass (*Poa pratensis* L.) and corn reared in the greenhouse were transplanted between May 12 to May 15, in two plots of 15 m × 20 m size. Twenty plants of each kind were randomly distributed, widely spaced, and plots repeatedly cultivated so as to obtain maximum aphid attraction (ORLOB & MEDLER, 1961). Aphids were first seen in the plots on May 19, and more frequently thereafter. After the intensity of aphid migration had been observed for some weeks, the one plot with the greater number of landings was selected for continuous recordings from May 19, to July 1, 1960.

Numbers of alate aphids that appeared to be feeding were determined by a careful sampling of all plants in the plot and the time these settlers remained on the plants was recorded in daily intervals thereafter. Position of a settled aphid on any part of the plant was marked by the pointing-direction and angle of inclination of a stake, stuck in the vicinity of the grass (Fig. 1; 3). If aphids moved to another part of the plant, the stake was moved to indicate the new feeding site. Aphids were considered to have flown off if they were no longer seen on the plant or found in a position that they could have reached by a short distance movement. Recording was discontinued when any doubt arose as to the identity of the aphid, whether it should be considered as a new arrival or as a colonizer with a previous history on the plant. Aphids in the process of walking, probing, or about to take-off were listed but not considered for marking. Further experimental procedures will be given under the appropriate context.

ON THE RELIABILITY OF THE MARKING METHOD

By using the method of marking aphids as described above, length of stay and movements of alatae cannot adequately be followed if migrants should move excessively once they have settled on the host. Unfortunately, little is known of the locomotion of alatae following alightment and subsequent feeding, although MÜLLER (1958) working with *Aphis fabae* Scop. has observed alatae and apterae to change place once or twice after the birth of the first or more nymphs.

To study the locomotory activities of alatae on the plant further, thereby testing the suitability of the marking method concurrently, the movements of migratory *R. padi-fitchii* were traced on potted oats placed in grassland on the campus of the University of New Brunswick, where the track of marked aphids on the plants could be checked regularly five times daily. Aphids that had been on the wing for some time but were of otherwise heterogenous experiences, were obtained from two wind traps similar to the one described by SHANDS (1942). These migrants were then placed on various parts of the plants as set out in Fig. 1. The initial feeding site was marked after aphids had been on the oats for about

30 min., during which time they usually probed several times. Some individuals took off again, but most aphids settled down for more prolonged feedings.

Aphids transferred to the test plants behaved quite like aphids found in feeding position on oats in the field. Some migrants left the host within 0—3 hr., while others stayed on for at least several hours (Fig. 1). The proportion of short stayers seemed to be less with alatae obtained from traps than with voluntarily settled aphids in the field (Tab. IV), probably because previous flights and a prolonged fasting period in the traps were important enough to strengthen the settling response (JOHNSON, 1958). Most migrants in Fig. 1; 1—7, 9—10, 13—18 did not move extensively but rather remained in the vicinity of the first site, hereby identifying themselves as specimens of the previous recordings. In other instances (Fig. 1; 8, 11, 12, 19, 20) aphids were more likely to move, for relatively long distances were covered by walking. On plants in the field and with voluntarily alighting aphids, a different pattern of movement may prevail, especially on densely tufted grasses where leaves and stems touch and intermingle.

Another premise assuring the reliability of the method of marking aphids is that the settling intensity must be sufficiently low so that the observer may differentiate between already recorded aphids and possible newcomers. Usually single plants were colonized by not more than 1 to 2 alatae at a time which could be easily distinguished, except on June 13 and 14, when flight activity was at its peak.

A third factor may have obscured settling behaviour of migrants as observed in the field. At times when the predator population had increased, some aphids were eaten, thereby eliminated from further recordings, and consequently listed as departers. Frequently, however, empty exuviae still adhered to the leaves and recording mistakes thus could be avoided. It is estimated that 1 to 3% of the settlers were eaten by ladybird beetles with a proportionally greater share among the long staying alatae.

Still other aphids may have dropped off, blown off, or died during the course of recording. All in all, these losses appeared negligible, with the exception of a drastic reduction of marked aphids following heavy rains on June 6. Although all these observations do not rule out a factor of uncertainty, sufficient reliance can be placed on the recording method if precautions are taken to exclude all doubtful cases from an evaluation. Furthermore, it is likely that possible errors affected recordings randomly and were not significantly different between the various plant species. Hence data gathered on the present occasion were considered reliable enough since they were wanted for comparative purpose only.

SPECIES COMPOSITION OF SETTLED MIGRANTS

As shown in Table I, most of the settled migrants were of the *R. padi-fitchii* complex. Oats carried the greatest number of these and also more *M. avenae* than the other species of grass. Only a few *R. maidis* and *M. avenae* were identified among settlers on *Phl. pratense* and *L. perenne*, while no record of these two species was obtained from *P. pratensis*. Unlike oats and grasses, *Z. mays* was visited by only a few migrants. Later in the season, *R. maidis* abounds on this host, which is, however, apparently not colonized in its early stages of growth.

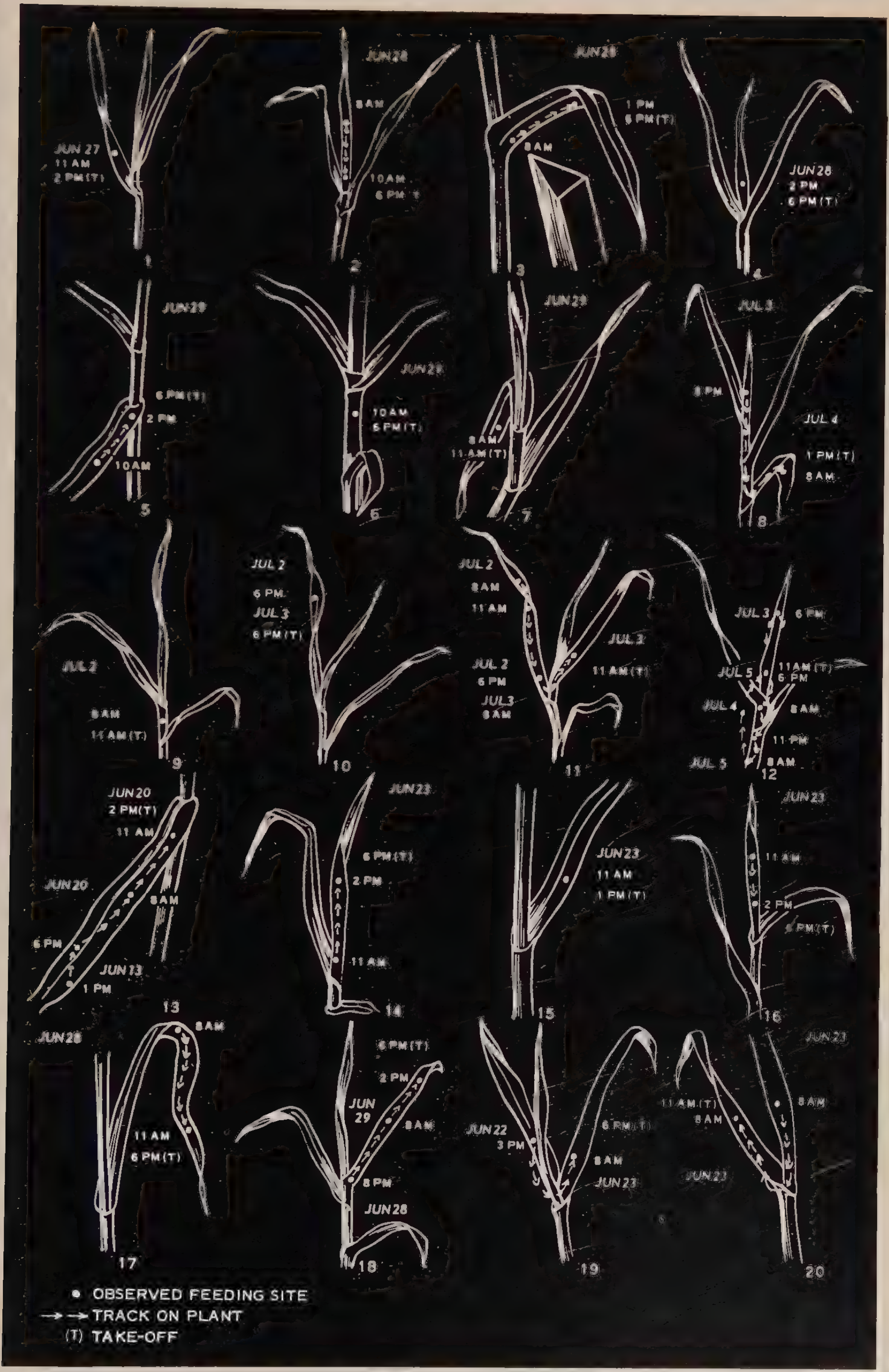

Fig. 1. Track and feeding sites of migratory *Rhopalosiphum padi-fitchii* released on oats.

R. maidis does not readily accept oats as a food plant and it had also little liking for the kind of grasses planted in the plot.

TABLE I

Number of cereal and non-cereal aphids found on 20 plants of each of 5 species of Gramineae and checked once daily from May 19, to July 1.

	Avena sativa	*Phleum pratense*	*Lolium perenne*	*Poa pratensis*	*Zea mays*
Rhopalosiphum padi-fitchii	277	208	212	142	2
Macrosiphum avenae	20	4	3	—	1
Rhopalosiphum maidis	1	4	5	—	—
Non-cereal aphids	3	3	4	1	1

A miscellany of non-cereal aphids were seen in the experimental plot (Tab. I). Alighters of these aphids were rarely engaged in continuous feeding of more than 2—3 min., but wandered about, probed briefly, and departed again. All test plants were cleared of non-cereal aphids within one hour after they were first noticed.

The consistently low numbers of *M. avenae* and *R. maidis* could have been due to a smaller aerial population of these species or may have been caused by an active avoidance of the test plants. Two wind-traps placed on the north end of the plot failed to give numerical evidence of a strikingly different composition of aphids in the air. However, as shown in Table I, *M. avenae* converged preferentially on oats which is known as a favourite host of this aphid as well as of *R. padi-fitchii*. Greatest numbers of *R. maidis* were found on *L. perenne*.

HOST PLANT PREFERENCE

The main objective of the present studies was to show any differences in the initial infestation pattern of alate cereal aphids on oats and grasses, because it is then that migrants are likely to be of greatest importance epidemiologically. MÜLLER (1958), and KENNEDY, BOOTH & KERSHAW (1959) have shown that the initial infestability of a host develops secondarily as a consequence of a differential rate of departure. Since flight activity — even at its peak — was too low to register alighting and departing aphids, the present observations did not take account of the initial phase of host plant selection by landing aphids. The above cited workers have also established that migratory aphids remain longer on preferred hosts than on less suitable plants although they landed on both kinds in about equal numbers. Host suitability may therefore well be measured in terms of the time alatae stayed on plants.

In Table II, the total number of days migrants remained on oats, grasses, and corn is given. For reasons already mentioned, it must be stressed again that aphid numbers in each category represent time values between an observed settling and an estimated departure, thus they do not relate to the absolute length of time aphids remained on the host. Obviously, a large part of marked settlers took off again from all plants within a day, but more migrants remained on oats than on either species of grass. In the following time intervals of 1 to 5 days the same relationship held true in that migrants stayed longer on oats than on grasses.

TABLE II

Length of time (in days) alate cereal aphids remained on 20 plants of various Gramineae when checked once daily from May 19, to July 1.

	Avena sativa		*Phleum pratense*		*Lolium perenne*		*Poa pratensis*		*Zea mays*
Time (Days)	T	P	T	P	T	P	T	P	T
<1	164	(61)	145	(79)	159	(84)	100	(84)	2
1—2	42	(16)	25	(14)	18	(10)	13	(11)	1
2—3	21	(8)	8	(5)	6	(4)	2	(2)	—
3—4	26	(10)	4	(2)	4	(2)	1	(1)	—
4—5	7	(3)	1	(.5)	2	(1)	2	(2)	—
>5	8	(3)	1	(.5)	1	(.5)	1	(1)	—
Recorded Landings	268	(100.)	184	(100.)	190	(100.)	119	(100.)	3
Total Landings	298		216		220		142		3

T: total numbers of aphids.
P: numbers of aphids in per cent of recorded landings.

Differences in the duration of stay on the three grasses as compared to oats were statistically significant, especially in the < 1 day and > 3 days period, but less so between 1 to 3 days (Tab. III).

TABLE III

P-values based on x^2 computed from pooled results of Table II and indicating differences in length of time cereal aphids remained on grasses as compared with the time spent on oats.

Time (Days)	*Phleum pratense*	*Lolium perenne*	*Poa pratensis*
<1	<0.02*)	<0.01*)	<0.02*)
1—3	>0.05	>0.05	<0.05*)
>3	<0.01*)	<0.01*)	<0.01*)

*) Significant difference.

There was some indication of an increased stay of migrants on *Phl. pratense* as compared with *L. perenne* and *P. pratensis*, at least in the < 1 to 4 days intervals, but these differences were not statistically significant. On corn, 21 migrants of *R. padi-fitchii*, and 2 of *M. avenae* were observed wandering about, probing, or departing; only 3 appeared to feed.

Table II also lists the total number of aphids found on the test plants and the total number of the recorded settlers; the difference consisting of aphids omitted from registration because of an uncertain history on the plants, predation, or death. On the basis of total recordings there was satisfactory evidence that migrants accumulated selectively on the various hosts. Aphid counts on *P. pratensis* were strikingly lower than on either grass or on oats, which was infested by the greatest number of migrants. From these data the test plants could be grouped in the series *A. sativa* > (*Phl. pratense*, *L. perenne*) > *P. pratensis* > *Z. mays*, which reflects the ecological value of plants as hosts of *R. padi-fitchii.*

The high proportion of short stayers revealed by the daily checking schedule (Tab. II) offered an opportunity to follow their rate of departure by recording movements of alatae hourly on the plant. Two days (June 22, 28) of favourable flight conditions were selected for this and alatae found on the plants were marked from 8 :00 a.m. to 8 :00 p.m. as described earlier. Again, many migrants departed shortly after they had been recorded, and more so from grasses than from oats (Tab. IV). Oats also supported the greatest number of migrants which remained longer on this host than on the grasses.

TABLE IV

Length of time (in hours) alate cereal aphids remained on 20 plants of various Gramineae when checked six times daily on June 22, and June 28.

Time (hours)	*Avena sativa*		*Phleum pratense*		*Lolium perenne*		*Poa pratensis*	
	T	P	T	P	T	P	T	P
<1	21	(23)	28	(41)	25	(36)	26	(46)
1—2	15	(16)	5	(7)	9	(12)	5	(9)
2—5	14	(15)	21	(30)	17	(24)	14	(24)
5—8	8	(9)	1	(1)	10	(14)	5	(9)
8—24	21	(23)	11	(16)	7	(10)	6	(10)
>24	13	(14)	3	(4)	2	(3)	1	(2)
Recorded Landings	93	(100)	69	(100)	70	(100)	57	(100)

T: total number of aphids
P: numbers of aphids in per cent of recorded landings.

These data are rather similar to those in Table II, although differences in settling and departing frequencies between the various test plants were less marked in the present observation period, possibly because the shorter time interval did not allow differences to manifest themselves as conspicuously. Furthermore, the present data were obtained from the more "dispersive" segment of the migrant population, i.e. aphids which predominantly stayed on the hosts for less than one day.

COLONIZATION OF GRAMINEAE

Population size and development is the final and probably most decisive criterium of host suitability. Hence larvi-position and total number of developing nymphs on all plants in the plot were determined three times during the observational period (Tab. V). Aphids were usually found on the lower side of the leaves, and care must be taken not to disturb the settled insects. Only 4 nymphs were found on corn, deposited by a single "worn-out" migrant of *R. padi-fitchii*, which by its appearance was unable to fly off again. All these aphids dispersed within two days and none were present after the second day. Table V shows that many more larvae were deposited on *P. pratensis*, and still more on *Phl. pratense* and *L. perenne*. Oats as in other experiments was the most suitable host on which largest populations developed. Results of these experiments are additional evidence of a selective infestability of grain and various grasses.

TABLE V

Total number of nymphs deposited by alate cereal aphids on 20 plants of each of five species of Gramineae from May 19, to June 28.

Counted on	*Avena sativa*	*Phleum pratense*	*Lolium perenne*	*Poa pratensis*	*Zea mays*
VI/11	285	87	54	31	4
VI/18	183	92	35	27	—
VI/28	399	112	62	36	—
Totals	897	291	151	94	—

The fact that the aphid population was reduced naturally must be called to attention once more. Ladybird beetles appeared in the plot in early June, increased in numbers throughout the month, and by early July aphid populations were reduced to such an extent that further recording became unreliable. Since greatest numbers of aphids were found on oats, it seems likely that predators took a heavier toll from this host than from grasses. Factors of different kinds certainly have contributed to the decline of populations in early July. KENNEDY & BOOTH (1951) have interpreted aphid taste as a synthesis of two selective forces, plant-taxonomic stimuli as expressed in characters of the plant species, and physiological stimuli as determined by conditions, especially age of a plant. Thus population peaks partly depend upon growth and development of the host plants. In July while maturing, oats apparently proceeds through stages of decreasing host suitability, and aphid numbers decline parallel to changes in host physiology. What little is known of the grass host ecology of cereal aphids, the present and previous observations (ORLOB & MEDLER, 1961) favour a similar decline in host suitability when grasses approach maturity.

WEATHER AND APHID MIGRATION

Since aphids are weak fliers, migration is often considered "a calm weather phenomenon" in which wind speed governs take-offs under otherwise suitable conditions of temperature and light (JOHNSON, 1954). JOHNSON (1954) and HAINE (1955), on the basis of experimental evidence stressed quite properly that wind speed may be less restrictive than often assumed, depending upon the species, seasonal form, and previous experience of the alatae involved.

In the present studies it was learned that migrational activity within a day's period never came to a complete standstill, in spite of prevailing conditions thought to prevent aphids from flying. On June 2, a day of frequent though light showers, new landings and take-offs were listed. Similarly, at times of appreciable winds (in gusts up to 20 m.p.h.) such as on June 17, and June 26, departing and alighting migrants were recorded. On the same occasion, two take-offs of *M. avenae* from rye were observed during a short calm after these aphids had made previously unsuccessful attempts to depart. Although observations like this cannot contradict the importance of metereological conditions (MÜLLER & UNGER, 1952) and other factors (JOHNSON, 1954) in governing the extent of aphid flight, the above examples show migration as a continuous process.

Most spring and summer days in Canada's Maritimes and certainly in other parts of the world are far from being ideal for active aphid dispersal. Yet aphids migrate, either "against" the day's weather or delay flying until conditions occur that are more favourable than the over-all weather pictures make us believe. In the experimental plot, considerable flight activity was noted in the morning and early evening hours, or even between rain showers.

DISCUSSION

Field observations have led several workers (NEWTON, PALMER & LIST, 1953; MÜLLER, 1958; KENNEDY *et al*, 1959) to postulate an extremely dispersive behaviour of migratory aphids. According to this view, migrants remain air-borne for some time, alight on various hosts, make a few probes, and take off again. These two alternating phases of feeding and dispersion seem to be the fundamental pattern in alate insects, governed by a neuro-muscular system which connects ingestion and locomotion antagonistically (THORSTEINSON, 1960).

JOHNSON (1954) has argued for a more sedentary habit of aphids which usually stay on a suitable host once they have alighted. Data of the present studies are in favour of a relatively restless behaviour of alate *R. padi-fitchii*, as it was indicated for *M. avenae* in previous work (ORLOB & MEDLER, 1961). Just how dispersive the host selection pattern of these aphids is, remains to be shown, since, unlike the observations of the English and German groups, flight density in my experiments was too low to allow for actual counts of alighting and departing aphids. With regard to length of time alighted aphids remained on a food plant, KENNEDY *et al* (1959, b) noted that 50% of alate *Brevicoryne brassicae* L. left field-exposed cabbage within 30 min. after plants were brought indoors, the length of stay being influenced by the time of the day alighting took place. In comparison only 23% of the settled alatae in the present studies left oats within one hour. Differences in the methods of observation are important enough to account for this discrepancy. Flight and settling behaviour, of course, may also vary with different species of aphids as it has been shown by KENNEDY (1950), and HAINE (1955).

The main interest in the present studies centered on food plant preference of a cereal aphid. THORSTEINSON (1960) in his excellent review of host plant selection in phytophagous insects concluded that visual signals generated from the plant do not contribute significantly to the identification of the plant, but rather that the food plant is selected by gustatory and possibly olfactory stimuli. With aphids, KENNEDY (1950) and KENNEDY *et al* (1959) have failed to observe an active host selection by approaching aphids which landed indiscriminately on host and non-host alike, although they seemed to avoid landings on plants drastically different in size and appearance from the host more often than on those displaying the habit of the food plant group. BODENHEIMER (1957) on the other hand has maintained that aphids are more directive in search for an appropriate host, admitting that orientation may be such as to lead only into the approximate vicinity of, rather than onto the host plant. MÜLLER & UNGER (1951) (c.f. BODENHEIMER, 1957) apparently have recognized a preferred alightment on beans susceptible to aphid infestation over that on resistant plants, but this was not observed in subsequent work (MÜLLER, 1958).

In the absence of any conclusive evidence, impressions gained in the present work by comparing ratios of cereal and non-cereal aphids seen in the plot, support the view that aphids are led (visually and olfactorily?) into the habitable neighbourhood of their food plants, "tasting out" differences within such a group. MÜLLER & HENNING (1960) have interpreted host selection, host colonization, and reproduction rate of aphids to be directed by the single principle of food quality which is perceived following prolonged feedings on the plant. Data of Table II in the present work could be explained in such terms when compared to those in Table IV; differences between the various hosts were better established through daily checking intervals than through hourly intervals.

In connection with an evaluation of the present results it must be considered that grasses used for recordings were young and first year seedlings which are more prone to aphid infestation than perennial grasses of advanced growth available in nature at the time of the experiment (KENNEDY & BOOTH, 1951). Thus conclusions derived from the present results are certainly on the favourable side.

Apart from behavioural studies, host selection and colonization assumes additional interest if the role of aphids in virus transmission is considered. KENNEDY *et al* (1959 b) have discussed implications of their findings in relation to the transmission of non-persistent viruses. This situation, however, is decisively different from the sequence of transmission involved in the case of persistent viruses. Based on greenhouse studies, such viruses are acquired in periods of several hours or even days, transmission to healthy plant is achieved within 1—2 hours, and virus is often retained for as long as the aphid lives. The epidemiological consequence of this is that at least one intimate feeding of about a day on the virus source is required for the migrants to become viruliferous. Hence important plants epiphytotically are those on which aphids stay long enough to pick up the virus, even though a prolonged sojourn on a suitable host may strengthen the subsequent settling response (JOHNSON, 1958), hereby reducing the number of aphids that could pass on the virus to healthy plants. This then means that the advantage of a permanent virus charge of aphids carrying persistent viruses is counteracted by their tendency for reduced dispersive activities which, on the other hand, is a prerequisite for the effective transmission of the quickly lost non-persistent viruses.

In these case of barley yellow dwarf virus many grasses have been incriminated as potential carriers of virus, primarily in greenhouse tests (c.f. ROCHOW, 1961). Although few implications can be drawn from these findings, several workers have suggested grasses as the main foci of yellow dwarf virus (c.f. ROCHOW, 1961), whereas others have reserved some doubt on this because aphids were rarely present on grasses (TOKO & BRUEHL, 1959; ORLOB & MEDLER, 1961). The present data have shown that three common grasses indeed were acceptable to *R. padi-fitchii* for periods sufficient to acquire virus, although fewer migrants settled on grasses than on oats and a greater proportion departed from the grasses in times below the minimum acquisition threshold. Given this system of quantitative host preference, KENNEDY's (1950) proposal, that plants more resistant to aphid infestation are the more important ones in virus epidemiology, cannot be maintained with regard to acquisition of persistent virus, true as it may be for non-persistent viruses or even for the quickly achieved transmission of a persistent virus.

Possible landings of cereal aphids on grasses and subsequent re-migration into grain fields is only one of many factors which in their entitety are responsible for the occurrence and propagation of the barley yellow dwarf virus disease. In the first place, virus in grasses must be available to the aphid (TOKO & BRUEHL, 1959), and infection density in grasses would need to be at a relatively high level to furnish the inoculum for the occasional visitors. The fact that a relatively small segment of the vector populations carries the virus (ORLOB, ARNY & MEDLER 1961) makes it necessary that populations of cereal aphids be high, which in fact seems to be true for many regions in North America. If in addition to this, transmission is geared to the proper vector specific strain of the virus (ROCHOW, 1961), the disease may occur in the endemic grass — grain cycle. As some viruliferous aphids diluted in the multi-taxic aeroplankton fan out over considerable distances, virus of unknown origin may be added to the local sources (ORLOB & MEDLER, 1961).

Another transmission system is the epidemic cycle in which virus infection goes beyond its average yearly occurrence. Evaluations of yellow dwarf epiphytotics in the U.S.A. have shown a close correlation to the size of the indigenous aphid populations or aphids introduced from outside sources (c.f. ROCHOW, 1961). One must however, warn against limiting consideration of virus epidemics to the one-sided look at aphid populations; undoubtedly various other factors contribute to outbreaks of this kind. Obviously, the final word on the mechanics of yellow dwarf epidemiology has to wait until the course of infection in natural insect and plant populations is better understood.

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ZUSAMMENFASSUNG

WIRTSPFLANZENWAHL VON GETREIDEBLATTLÄUSEN IM FREILAND IM BEZIEHUNG ZUR ÖKOLOGIE DER GELBVERZWERGUNGSVIROSE DER GERSTE

Die Besiedlung gewisser Gräserarten durch geflügelte Blattläuse ist verschiedentlich als Ausgangspunkt von Epidemien der Gelbverzwergungsvirose der Gerste (Rotblättrigkeit des Hafers) diskutiert worden. Ziel der vorliegenden Arbeit war es daher, den unterschiedlichen Befall von Hafer, deutschem Weidelgras, Wiesenlieschgras, Wiesenrispengras und Mais durch Blattläuse des *Rhopalosiphum padi-fitchii* Komplexes täglich oder mehrstündlich in einem Freilandbeet abzulesen. Dabei zeigte es sich, dass der Hafer von den Blattläusen vorgezogen wurde und es auf diesem Wirt zur Ausbildung grösserer Kolonien kam als auf den Gräserarten, während Mais überhaupt nicht besiedelt wurde. Wirtspflanzenpräferenz manifestierte sich ausserdem dadurch, dass die gelandeten Geflügelten längere Zeit auf Hafer verbrachten als auf den offensichtlich weniger geeigneten Gräsern. Die Befunde werden im Zusammenhang mit der Epidemiologie der Gelbverzwergung besprochen.

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AMINOSÄUREN IM SPEICHEL UND HONIGTAU DER GRÜNEN APFELBLATTLAUS *APHIS POMI* DEG.-HOMOPTERA

VON

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Im Speichel von *Aphis pomi* wurden fünf Aminosäuren und zwei nicht identifizierte ninhydrin-positive Verbindungen papierchromatographisch nachgewiesen. Der Honigtau enthält zwanzig Aminosäuren und in besonders hoher Konzentration die Amide Asparagin und Glutamin. Herkunft und Bedeutung der Aminosäuren und Amide in diesen Ausscheidungsprodukten werden diskutiert.

Die Wechselbeziehungen zwischen pflanzensaugenden Insekten und ihren Wirten sind dadurch charakterisiert, daß die Insekten mit ihren stechend-saugenden Mundwerkzeugen Nahrung aus dem pflanzlichen Gewebe aufnehmen und andererseits das Sekret ihrer Speicheldrüsen in das Gewebe abgeben. Dem Speichel kommt dabei eine Schlüsselstellung zu, denn er vermittelt offenbar nicht nur die Nahrungsaufnahme, sondern kann darüber hinaus die Ursache für pathologische Veränderungen in den Pflanzen sein. Bei gallbildenden Insekten sind diese Veränderungen zugleich die Voraussetzung für die weitere Nahrungsaufnahme. Für resistenzphysiologische Untersuchungen gewinnt damit die chemische Analyse der Speichelinhaltsstoffe besondere Bedeutung.

In der vorliegenden Arbeit wird versucht, die ninhydrin-positiven Substanzen im Speichel von *Aphis pomi* zu erfassen, während die Untersuchung des Honigtaues die Befunde der Speichelanalyse nur ergänzen soll.

MATERIAL UND METHODE

Für alle Untersuchungen wurden ungeflügelte Fundatrigenien der grünen Apfelblattlaus *Aphis pomi* Deg. verwendet. Es handelt sich stets um Freilandmaterial, das kurz vor Versuchsbeginn ins Laboratorium geholt wurde. Besonders vorteilhaft ist es, die Untersuchungen in den Monaten Juli, August und September durchzuführen, zu einem Zeitpunkt also, nachdem die wirtswechselnden Aphiden (*Rhopalosiphum insertum* (Walk.), *Sappaphis mali* Ferr.) den Apfelbaum bereits verlassen haben. Im Frühjahr und Frühsommer treten *Aphis pomi* und die wirtswechselnden Arten häufig in Kolonien vermischt auf.

Um den reinen Speichel von *Aphis pomi* zu gewinnen, wurden verschiedene Möglichkeiten geprüft. Am besten hat sich eine kegelförmige „Aphidenkammer" bewährt, die schnell und einfach herzustellen ist. Es wird aus einer runden Chromatographiepapier-Scheibe ein schmaler Sektor ausgeschnitten und das Chromatographiepapier mit einem Perlonfaden zu einem flachen Kegel zusammengeheftet. Anschließend wird für diesen Kegel ein passender Boden angefertigt. Dazu ist schwarzes Filtrierpapier, das zur Versteifung auf einer Kunststoffscheibe aufliegt, besonders geeignet. In der Mitte des Bodens wird ein Perlonfaden befestigt, der durch die Spitze des Kegels gezogen wird und diesen mit dem Boden verbindet (Abb. 1). Der Kegel muß nun an dem Perlonfaden nach unten über den Boden geschoben werden und die „Aphidenkammer" ist fertig. Es ist vorteilhaft, das Chromatographiepapier des Kegels mit destilliertem Wasser schwach anzufeuchten.

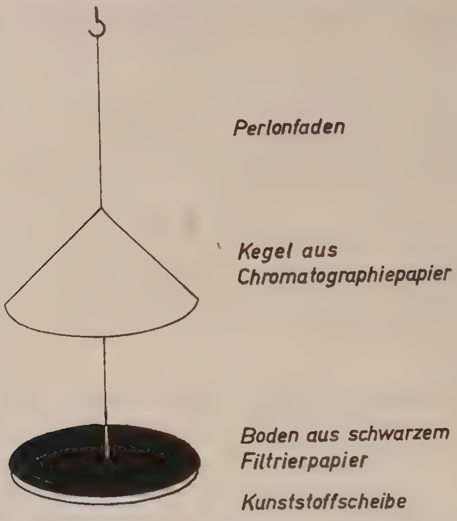
Perlonfaden
Kegel aus Chromatographiepapier
Boden aus schwarzem Filtrierpapier
Kunststoffscheibe

Abb. 1. Bestandteile einer „Aphidenkammer". Erklärungen im Text.

Nachdem diese „Aphidenkammer" vorbereitet ist, werden die Aphiden vorsichtig mit einem feinhaarigen Pinsel von den abgeschnittenen Zweigen ihrer Wirtspflanzen gelöst und etwa hundert Tiere auf den Boden des Chromatographiepapier-Kegels übertragen. Anschließend muß der angefeuchtete Papierkegel sofort lückenlos über den Boden geschoben werden, so daß die Aphiden eingeschlossen sind. Diese „Aphidenkammer" wird nun mit dem Perlonfaden unter zwei Leuchtstoffröhren so aufgehängt, daß sie gleichzeitig von einem zylindrischen Glasgefäß umschlossen ist (Abb. 2). Die Öffnung des Glasgefäßes wird mit zwei gelb-grünen Glasscheiben abgedeckt und die Gefäßwand mit schwarzem Papier ausgelegt, um seitlichen Lichteinfall zu vermeiden.

Das Licht der Leuchtstoffröhren fällt durch die gelb-grünen Glasscheiben1)) auf den Papierkegel (gelb-grünes Glas wurde in Anlehnung an die Versuche von Moericke (1950) verwendet) und lockt die Aphiden vom schwarzen Boden zum Chromatographiepapier. Dort angelangt, stechen sie wiederholt in das Papier ein und geben dabei nach Beobachtungen von Kloft (1956, 1960) und Kloft & Ehrhardt (1959) Speichel ab. Die Einstichfreudigkeit wird erhöht, wenn man

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1)) Es wurde handelsübliches Farbglas ohne genau bekannten Spektralbereich verwendet.

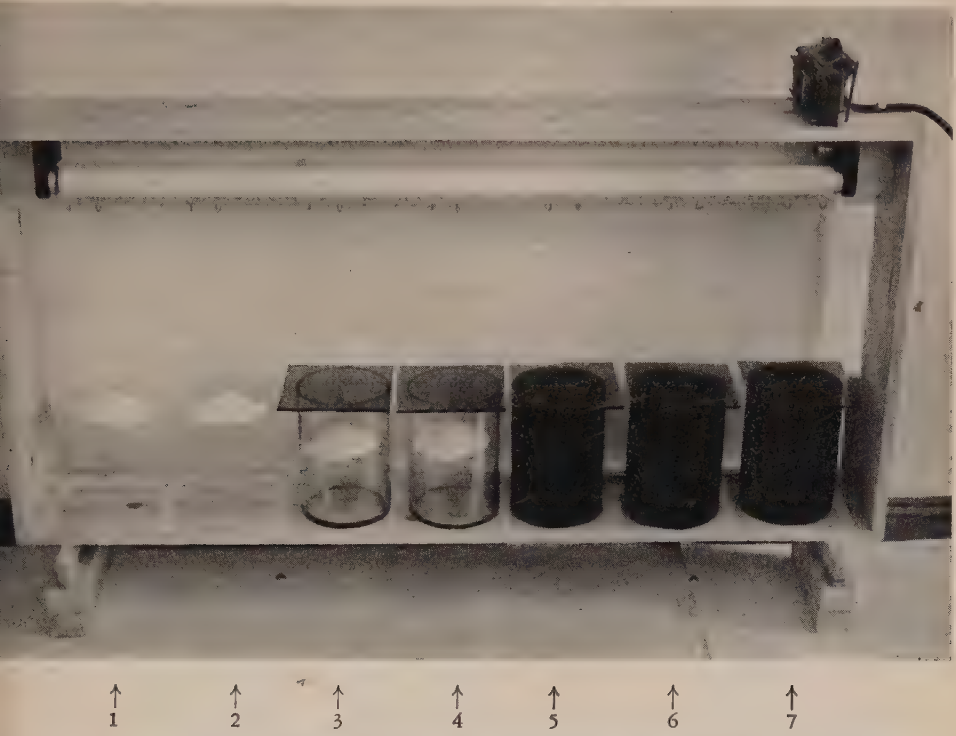

Abb. 2. Aufbau der Versuchsanordnung zur Anreicherung des Speichels im Chromatographiepapier.
1 u. 2 : Zwei „Aphidenkammern" ohne Glasgefäße und ohne gelb-grünen Glasscheiben.
3 u. 4 : Zwei „Aphidenkammern" in Glasgefäßen, deren Öffnung mit gelb-grünen Glasscheiben verschlossen ist. Die Glasgefäße sind noch nicht mit schwarzem Papier ausgelegt.
5, 6 u. 7: Drei vollständige Versuchsanordnungen.
Weitere Erklärungen im Text.

darauf achtet, daß das Chromatographiepapier stets schwach angefeuchtet bleibt. Die dazu notwendige hohe Luftfeuchtigkeit kann in den Glasgefäßen leicht erreicht werden, wenn man mit einem Handzerstäuber geringe Mengen destilliertes Wasser versprüht. Nach etwa fünf Stunden werden die Versuche abgebrochen.

Die Grundlage dieser Methode bilden demnach die Beobachtungen, daß *Aphis pomi* positiv phototaktisch reagiert und bevorzugt bei gelb-grünem Licht in angefeuchtetes Chromatographiepapier einsticht.

Nach den bisherigen Ergebnissen ermöglicht diese Methode Speicheluntersuchungen, ohne Verunreinigungen durch Honigtau befürchten zu müssen. Die Exkrete fallen, da die Tiere an der Innenseite des flachen Kegels hängen, nach unten auf den Boden und werden dort vom schwarzen Filtrierpapier aufgesogen. Wesentlich ist ferner, daß die Honigtauabgabe schon kurze Zeit später, nachdem die Aphiden von der Wirtspflanze abgenommen wurden, aussetzt.

Außerdem berühren die Aphiden das Chromatographiepapier nur mit den Tarsen. Sie laufen selbst vom Boden des Kegels zum Chromatographiepapier und nach Abschluß des Versuches selbst von dort wieder weg. Das ist notwendig, weil die Körperoberfläche von *Aphis pomi* mit Honigtau verunreinigt sein kann. Überträgt man die Tiere unmittelbar von der Wirtspflanze auf den Chromatographiepapier-Kegel, so erhält man auch ohne Aphiden-Einstich an der Auftragestelle stets merkbare positive Ninhydrinreaktion.

Das Chromatographiepapier darf bei allen diesen Arbeiten selbstverständlich nur mit sauberen Pinzetten oder Gummihandschuhen berührt werden. Die Ausscheidungen der Haut enthalten Aminosäuren und würden die Untersuchungen stören.

Für jede Speichelanalyse wurden sieben bis zehn derartige „Aphidenkammern" angesetzt. Sobald der Versuch beendet war, wurden die Speichelinhaltsstoffe mit 70%-igem Alkohol aus dem Chromatographiepapier eluiert, das Eluat filtriert und unter vermindertem Druck bei Zimmertemperatur bis zur Trockene eingeengt. Mit 1 ml 70%-igem Alkohol wurde das Eluat wieder aufgenommen und quantitativ auf den Startpunkt eines zweidimensionalen Chromatogramms aufgetragen. Infolge der vielen hundert Einstiche in das Chromatographiepapier und der damit verbundenen Speichelabgabe gelang es, ausreichende Ausgangskonzentrationen für die Analyse der ninhydrin-positiven Substanzen zu gewinnen. Als Lösungsmittelgemisch diente in der ersten Phase Butanol : Ameisensäure : Wasser = 75 : 15 : 10 und in der zweiten Phase Phenol : Wasser = 80 : 20. Mit diesen Gemischen wurde die günstigste Verteilung der Aminosäuren und Amide erreicht. Wichtig ist, daß anschließend an die zweite Phase das Phenol im warmen Luftstrom weitgehend aus den Chromatogrammen entfernt wird, denn die Ninhydrin-Farbreaktion ist um so schwächer, je höher der Phenolgehalt des Papieres ist. Bei den geringen Aminosäurekonzentrationen, wie sie bei den Speichelanalysen chromatographiert werden, ist das besonders wichtig.

Zum Nachweis der Aminosäuren wurden die Chromatogramme durch eine 0,2%-ige Ninhydrin-Azeton-Lösung gezogen und bei 60° zur Farbvertiefung ½ Stunde getrocknet.

Die Gewinnung des Honigtaues von *Aphis pomi* ist wesentlich einfacher. Es werden stark befallene, eingetopfte Apfelbäumchen ins Gewächshaus gebracht, auf Tischen waagerecht gelegt und unter die *Aphis pomi*-Kolonien sorgfältig gereinigte Glasscheiben gestellt. Der Honigtau wird so reichlich ausgeschieden, daß man auf den Glasscheiben mühelos brauchbare Ausgangskonzentrationen für die papierchromatographische Analyse sammeln kann. Beabsichtigt waren nur qualitative Untersuchungen. Es konnte deshalb auf genaue Wägungen der Honigtaumengen pro Analysengang verzichtet werden. Die Glasscheiben mit den Honigtautröpfchen werden mit 70%igem Alkohol abgewaschen und die Waschflüssigkeit samt gelöstem Honigtau durch eine Ionenaustauscher-Säule (Merk I) geschickt. Damit werden vor allem die reichlich vorhandenen Zucker abgetrennt, die die Chromatographie der ninhydrin-positiven Verbindungen stören. Sie laufen durch die Säule hindurch, während die Aminosäuren und Amide festgehalten werden. Um diese Verbindungen zu eluieren, wird eine 1n Ammoniaklösung in 70%igem Alkohol durch die Austauschersäule geschickt. Geringe Verluste, besonders bei Taurin, sind dabei zu berücksichtigen. Anschließend wird die ammoniakalische Lösung wie das Speicheleluat aus den Chromatographiepapier-Kegeln weiter verarbeitet (s. oben).

ERGEBNISSE

Im Verlauf der Untersuchungen wurden acht Speichelanalysen durchgeführt. Die nachgewiesenen Aminosäuren und deren Verteilung auf dem zweidimensionalen Chromatogramm zeigt Abb. 3.

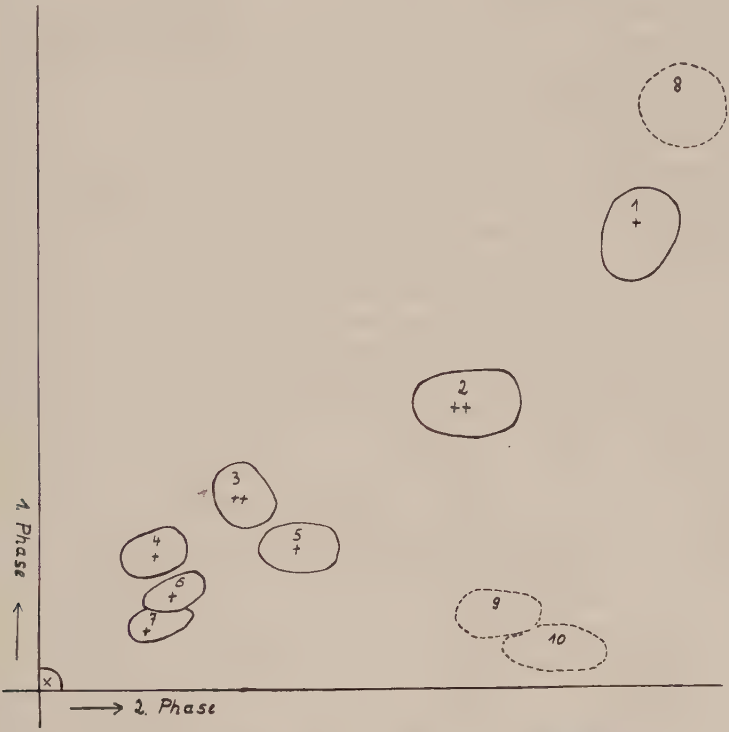

Abb. 3. Aminosäuren im Speichel von *Aphis pomi*.
1. Phase: Butanol : Ameisensäure : Wasser = 75 : 15 : 10; 2. Phase: Phenol : Wasser = 80 : 20. 1 = Valin, 2 = Alanin, 3 = Glutaminsäure, 4 = Asparaginsäure, 5 = Serin, 6 = nicht identifizierte Verbindung X1, 7 = nicht identifizierte Verbindung X2, 8 = Leucin, 9 = Arginin (?), 10 = Histidin, ---- = nicht sicher nachgewiesene Aminosäuren. Erklärung der Angaben über die Aminosäurekonzentration (+, ++) im Text.

Da auf eine quantitative Bestimmung der Aminosäuren zunächst noch verzichtet werden mußte, diente als Ersatz eine halbquantitative Bewertung der Aminosäurekonzentration, bei der die Farbintensität und Größe der Flecken auf dem Papier abgeschätzt wurde. Die Bewertung erfolgte in fünf willkürlich gewählten Größenklassen (+, ++, +++, ++++, +++++), wobei „+" die geringste Konzentration bedeutet. Im Speichel treten nur die beiden niedrigsten Konzentrationen auf, während im Honigtau von *Aphis pomi* die Aminosäuren und Amide in allen Bewertungsklassen vorliegen.

Alanin und Glutaminsäure sind im Speichel von *Aphis pomi* am stärksten vertreten. In geringerer Konzentration kommen noch regelmäßig Asparaginsäure, Valin, Serin und zwei nicht identifizierte Verbindungen vor. Die Rf-Werte der unbekannten Verbindungen liegen in der ersten und in der zweiten Phase unter 0,2 (vergl. Abb. 3). Cystin, Cystein und Cysteinsäure scheiden auf Grund von Vergleichschromatogrammen mit Reinsubstanzen aus. Im Honigtau von *Aphis pomi* konnten die beiden unbekannten Verbindungen nicht festgestellt werden.

Außer diesen Aminosäuren wurden bei einigen Analysen noch Spuren von Leucin, Histidin und wahrscheinlich Arginin gefunden (gestrichelte Orte der Abb. 3). Gesichert ist zwar damit noch nicht, daß diese Aminosäuren im Speichel vorkommen, doch ist die Ansicht berechtigt, daß bei weiterer Verbesserung der Methode und Erhöhung der Ausgangskonzentration noch mehr Aminosäuren nachgewiesen werden können.

Im Verlauf der Speicheluntersuchungen wurde besonders darauf geachtet, daß keine Verunreinigungen des Chromatographiepapiers durch Honigtau vorkommen können. Der Honigtau enthält nach den Befunden von MALTAIS & AUCLAIR (1952), GRAY (1952), MITTLER (1953), AUCLAIR (1958), LAMB (1959) und nach den Ergebnissen der eigenen Analysen so hohe Aminosäure- und Amid-Konzentrationen, daß selbst geringe Mengen die Speicheluntersuchungen mit der angewandten Methode unmöglich machen würden. Die Untersuchung des Honigtaues auf Aminosäuren und Amide war deswegen erforderlich und ein Vergleich mit den Aminosäuren im Speichel mußte besonders aufschlußreich sein.

In Tab. I sind die Ergebnisse der Honigtauanalysen von *Aphis pomi* zusammengestellt.

TABELLE I

Aminosäure und Amide im Honigtau von Aphis pomi

	a
Asparagin	+++++
Glutamin	+++++
Threonin	++++
Serin	++++
Glutaminsäure	++++
Asparaginsäure	+++
Leucin/Isoleucin	+++
Valin	+++
Alanin	+++
Glycin	+++
Lysin	+++
Histidin	++
Prolin	++
Oxyprolin	++
Phenylalanin	++
Tryptophan	+
Tyrosin	+
Homoserin	+
γ-Aminobuttersäure	+
Methioninsulfoxyd	+
Oxydationsprodukte des Cystein	+
Taurin (?)	+

a: Größenklasse der Flecken.
+ = niedrigste Konzentration.
+++++ = höchste Konzentration.

Insgesamt konnten zweiundzwanzig Aminosäuren und Amide nachgewiesen werden. Die beiden Amide Asparagin und Glutamin treten am stärksten auf. Die Konzentration der Amide war bei einigen Analysen derart hoch, daß die chromatographische Verteilung der Aminosäuren gestört wurde. Von den Aminosäuren liegen vor allem Threonin, Serin und Glutaminsäure in hohen Konzentrationen vor.

Dieses Analysenergebnis stimmt gut mit den Angaben über den Aminosäure- und Amidgehalt des Honigtaues anderer Homopteren überein. So fanden MALTAIS & AUCLAIR (1952) 22 ninhydrin-positive Verbindungen im Honigtau von *Myzus circumflexus* (Buck.), GRAY (1952) 19 Verbindungen im Honigtau von *Pseudococcus brevipes* (Ckll.), MITTLER (1953) 12 Verbindungen im Honigtau von *Tuberolachnus salignus* (Gmelin), AUCLAIR (1958) 19 Verbindungen im Honigtau von *Acyrthosiphon pisum* (Harr.) (= *Acyrthosiphon onobrychis* (B.d.F.) = *Aphis pisi* Kalt.) und schließlich LAMB (1959) 18 Verbindungen im Honigtau von *Brevicoryne brassicae* (L.). Es handelt sich dabei fast immer um die gleichen Aminosäuren und Amide, nur die Konzentration der Verbindungen ist bei den Homopteren-Arten sehr unterschiedlich. Eine Ausnahme sind Asparagin und Glutamin, die bei den meisten Untersuchungsobjekten auffallend stark auftreten.

Ob sich die Zusammensetzung des Honigtaues in Abhängigkeit von der Jahreszeit verändert, wurde nicht geprüft. Für die vorliegende Untersuchung war dies auch unwesentlich. Der Honigtau und der Speichel wurden stets zur gleichen Zeit gesammelt, weshalb die Analysenergebnisse unmittelbar vergleichbar sind.

Bis auf die beiden nicht identifizierten Verbindungen kommen sämtliche Aminosäuren, die im Sekret der Speicheldrüsen auftreten, auch im Honigtau vor. Das könnte zu der Schlußfolgerung führen, daß es sich bei den im Speichel nachgewiesenen Aminosäuren trotz aller Vorsicht lediglich um Verunreinigungen durch Honigtau handelt. Dagegen spricht aber eindeutig, daß die Amide Asparagin und Glutamin und auch die Aminosäure Threonin bei den Speichelanalysen nicht gefunden wurden, obwohl gerade diese Verbindungen im Honigtau in höchsten Konzentrationen vorliegen. Wenn Verunreinigungen durch Honigtau vorkommen, dann muß damit gerechnet werden, daß diese Verbindungen zuerst in den Chromatogrammen der Speichelanalysen störend auftreten.

DISKUSSION

Aphis pomi verursacht bei Massenbefall an jungen Trieben des Apfelbaumes Stauchungen und an den Blättern Rollungen. Die Schäden der Wirtspflanze werden zweifellos durch die Inhaltsstoffe des Speichels verursacht, weil der Schädigungsgrad der Befallsstärke unmittelbar parallel läuft und die Pflanzen bei rechtzeitiger Entfernung der Aphiden normal weiterwachsen.

Von den bisher im Speichel pflanzensaugender Insekten vermuteten oder nachgewiesenen Substanzen hat man vor allem die β-Indolylessigsäure (NYSTERAKIS, 1946, 1948, 1948a, 1948b; BOYSEN-JENSEN, 1948; HOPP, 1955, 1958 u.a.) und in neuerer Zeit freie Aminosäuren für die phytopathologische Wirkung verantwortlich gemacht. Die Aufmerksamkeit wurde auf die freien Aminosäuren gelenkt, als durch die Untersuchungen von COMMONER & NEHARI (1953), STEINBERG (1949), STEINBERG, BOWLING & MCMURTREY (1950), VIRTANEN &

LINKOLA (1957) und MIETTINEN & WARIS (1958) bekannt wurde, welchen großen Einfluß freie Aminosäuren in bestimmten Konzentrationen auf das pflanzliche Wachstum ausüben können. Besonderes Gewicht erhielt diese Tatsache, als der Nachweis freier Aminosäuren im Speichel pflanzensaugender Insekten gelang (KLOFT, 1956, 1960; KLOFT & EHRHARDT, 1959; ANDERS, 1957, 1958; SCHÄLLER, 1960).

Eine eingehende Darstellung der Rolle der Aminosäuren im Wechselspiel zwischen pflanzensaugenden Insekten und dem Wirtsgewebe geben KLOFT (1956, 1960) und HENKE (1961). Im Zusammenhang mit den Ergebnissen der vorliegenden Arbeit sollen deswegen nur einige Gedanken zum Aminosäuregehalt des Speichels der Hemipteren diskutiert werden.

Der Nachweis der freien Aminosäuren im Speichel erfolgte bei allen Untersuchungen papierchromatographisch. Unterschiedlich waren dagegen die Methoden zur Anreicherung des Speichels. Man nützte vor allem die Tatsache aus, daß die Hemipteren den Speichel nicht nur in pflanzliches Gewebe abgeben. Setzt man die Pflanzensauger z.B. auf eine Glas- oder Holzfläche, auf verfestigte Gelatine, oder Agar-Agar, auf angefeuchtetes Papier, oder eine Kollodium-Membran usw., so versuchen sie einzustechen und geben dabei Speichel ab (MOERICKE, 1950; GOODCHILD, 1952; KLOFT, 1956, 1960; KLOFT & EHRHARDT, 1959; MILES, 1958, 1959, 1959a; SCHMIDT, 1959; SCHÄLLER, 1960; u.a.). Zur Anreicherung des Speichels für analytische Zwecke hat sich bisher von diesen Möglichkeiten angefeuchtetes Chromatographierpapier am besten bewährt.

Dreht man die Tiere auf den Rücken, so kann man gleichfalls beobachten, wie kurze Zeit später an der Spitze der Stechborsten ein winziges Speicheltröpfchen austritt (BRAUN & MARAMOROSCH, 1951; ANDERS, 1957, 1958). Diese Tröpfchen können abgesammelt werden und gleichfalls als Ausgangsmaterial zur Speichelanalyse dienen. Bei größeren Insekten kann das Speicheldrüsensekret auch gewonnen werden, indem man die Drüsen aus dem Tier herauspräpariert, die Drüse anritzt und das Sekret ausfließen läßt (KLOFT, 1956, 1960; u.a.).

Welche dieser Methoden angewandt werden kann, richtet sich vor allem nach der Größe des Untersuchungsobjektes. Fraglich ist dabei, ob die Methode der Speichelanreicherung irgend einen Einfluß auf das Analysenergebnis hat. Untersuchungen liegen darüber noch nicht vor.

MILES (1958, 1959, 1959a, 1960, 1960a) vertiefte diese Beobachtungen, indem er nachweisen konnte, daß von den Pflanzensaugern zwei verschiedene Speichelsorten ausgeschieden werden — ein wässriges Sekret und ein Sekret, das bald nach dem Austritt aus den Stechborsten zähflüssig wird und schließlich zu einer festen Masse gerinnt. Miles sieht, sicherlich zu Recht, in dem sich verfestigenden Sekret das Material für die Speichelscheide, während das wässrige Sekret für die enzymatische Spaltung der Nahrung verantwortlich gemacht wird und den Pflanzensaugern die chemischen Reize der Nahrung vermittelt. Die Untersuchung beider Speichelsorten von *Oncopeltus fasciatus* (Dall.) ergab, daß das Material für die Speichelscheide vor allem aus einem Lipoprotein besteht, das reich an Tyrosin ist und Spuren von Tryptophan enthält, während in der wässrigen Speichelsorte ein Mucoprotein und eine Amylase nachgewiesen werden konnten. Außerdem wurden in den Speicheldrüsen von *Oncopeltus fasciatus* noch verschiedene Carbohydrasen und nach den Befunden von BRONSKILL, SALKELD &

FRIEND (1958) eine Proteinase und eine Lipase gefunden. Mit welcher Speichelsorte diese Enzyme ausgeschieden werden, ist noch ungeklärt, doch ist anzunehmen, daß sie mit dem wässrigen Speichel abgegeben werden. Auf Grund der unterschiedlichen Funktionen der beiden Speichelsorten liegt außerdem die Vermutung nahe, daß auch die freien Aminosäuren und andere phytopathologisch wirksame Verbindungen in dem wässrigen Speichel vorkommen.

Die folgende Tabelle gibt eine Übersicht über die bisher im Speichel von Pflanzensaugern nachgewiesenen freien Aminosäuren und Amide.

TABELLE II

Aminosäuren und Amide im Speichel von Homopteren und Heteropteren

Autor:	Kloft	Kloft	Kloft	Kloft Ehrhardt	Anders	Schäller	Schäller
Objekt:	P. apter. 1)	P. pras.	M. ascal.	L. abiet.	V. vitif.	V. vitif.	A. pomi
Alanin	+	+	+	+		+	+
Glutaminsäure	+	+	+		(+)	+	+
Asparaginsäure	+	+	+	+		(+)	+
Serin	+	+		+		+	+
Arginin	+	+		+		+	(+)
Valin	+			+	(+)	(+)	+
Glycin		(+)	+			+	
Cystin	+			+			
Lysin	+	+			+		
Threonin	+					(+)	
Asparagin						+	
Glutamin						+	
Histidin	+				+		(+)
Prolin	+						
Tyrosin	+					(+)	
Methionin	+						
Tryptophan					+		
Leucin				+			(+)
n. indent. Verb. des Reblaussp.						+	
n. ident. Verb. X1							+
n. ident. Verb. X2							+

+ = nachgewiesene Verbindung
(+) = nicht sicher nachgewiesene Verbindung
1) Vollständige Artnamen der Untersuchungsobjekte: *Pyrrhocoris apterus* L. (Heteroptera); *Palomena prasina* L. (Heteroptera); *Myzus ascalonicus* Donc. (Homoptera); *Liosomaphis abietina* (Walk.) (Homoptera); *Viteus* (*Phylloxera*) *vitifolii* Shim. (Homoptera); *Aphis pomi* Deg. (Homoptera).

Diese Befunde sind noch sehr lückenhaft und dürfen auf keinen Fall verallgemeinert werden. Bei der Analyse des Speichels anderer Hemipteren können ganz andere Aminosäure-Kombinationen gefunden werden. Trotzdem verdient die Tatsache Beachtung, daß Asparaginsäure, Glutaminsäure, Alanin, Serin, Arginin und Valin bei fast allen Untersuchungsobjekten nachgewiesen wurden. Bei der Mehrzahl der Speichelanalysen wurde außerdem die Aminosäure-Konzentration nach der Fleckengröße und der Intensität der Farbreaktion mit Ninhydrin auf dem

Chromatographiepapier geschätzt. Dabei zeigte sich, daß im allgemeinen Glutaminsäure und Alanin die anderen Verbindungen in der Konzentration wesentlich übertreffen. Eine Ausnahme stellt nach den Befunden von SCHÄLLER (1960) der Speichel erwachsener Blatt-Rebläuse dar. Asparagin und Glutamin liegen darin im Vergleich zu den ebenfalls gefundenen Aminosäuren Alanin, Serin, Glycin, Arginin und Glutaminsäure in zwei- bis dreifach höherer Konzentration vor. Verunreinigungen durch Honigtau scheiden bei diesem Untersuchungsobjekt aus, weil es bei den erwachsenen Rebläusen zum Verschluß der Afteröffnung kommt. Diese Tatsache erklärt möglicherweise auch den hohen Amidgehalt des Speichels, denn normalerweise sind die Amide bei Aphiden im Honigtau anzutreffen (MALTAIS & AUCLAIR, 1952; GRAY, 1952; MITTLER, 1953; AUCLAIR, 1958; LAMB, 1959; u.a.). Sie stellen sogar bei bestimmten Aphiden das Hauptausscheidungsprodukt des Stickstoff-Stoffwechsels dar. Bei der Reblaus fehlt diese Möglichkeit der Stickstoffausscheidung, weshalb offenbar der Speichel für die Ausscheidung von Exkreten mit verantwortlich ist.

Diese Auffassung wird durch die Ergebnisse der Speichelanalyse von *Aphis pomi* — einem starken Honigtauausscheider — bestätigt. Amide fehlen im Speichel von *Aphis pomi* vollständig. Dafür treten sie in besonders hoher Konzentration in Honigtau auf (Tab. I).

Berücksichtigt werden muß bei diesen Untersuchungen, daß die Zusammensetzung des Speichels möglicherweise Schwankungen unterworfen ist. Durch die Arbeiten von CARTER (1951) und NUORTEVA (1955, 1958, 1958a, 1959) ist bekannt geworden, daß die Toxizität eines Pflanzensaugers gegenüber einer Wirtspflanze stark von der vorher aufgenommenen Nahrung abhängen kann. Daraus wurde der Schluß gezogen, daß die phytotoxischen Substanzen im Speichel wenigstens zum Teil von den Wirtspflanzen stammen und daß mit dem Wechsel der Wirtspflanze auch die Speichelzusammensetzung wechselt. Es handelt sich möglicherweise um Spaltprodukte der Nahrung, die mit dem Speichel in toxischen Konzentrationen wieder in die Pflanze abgegeben werden.

Diese Auffassung wird auch von ANDERS (1957, 1958) im Zusammenhang mit dem Aminosäuregehalt des Reblausspeichels vertreten. Die Aminosäuren im Speichel würden demnach Spaltprodukte der eiweißhaltigen Nahrung darstellen. Sie sind nach den Befunden von Anders unmittelbar für die Gallbildung verantwortlich.

Auf die große Bedeutung der Aminosäuren im Speichel pflanzensaugender Insekten weist auch folgender Versuch von NUORTEVA (1959) hin. Werden der Zikade *Calligypona pellucida* F. bei künstlicher Ernährung zusätzlich die für die Ernährung entbehrlichen Aminosäuren Valin oder Glutaminsäure gegeben, so verursachen diese Aminosäuren offenbar über den Speichel eine Erhöhung der Phytotoxizität. Wurde der Nährlösung die essentielle Aminosäure Histidin zugesetzt, so zeigte sich kein solcher Effekt.

Diese Beobachtung von NUORTEVA gewinnt an Bedeutung, wenn man bedenkt, daß Glutaminsäure und Valin im Speichel der meisten bisher untersuchten Pflanzensauger nachgewiesen werden konnte (Tab. II). Glutaminsäure tritt im Speichel sogar in relativ hoher Konzentration auf. Es ergibt sich also eine auffallende Übereinstimmung zwischen der beobachteten Wirkung von Glutaminsäure und Valin, nachdem sie der künstlichen Nährlösung zugesetzt wurden, und dem regelmäßigen Auftreten dieser Aminosäuren im Speichel der untersuchten Pflanzensauger, die alle mehr oder weniger phytotoxisch wirksam sind. Histidin verursacht diese Wirkung nicht und wurde bisher auch nur im Speichel der Wanze *Pyrrhocoris apterus* L. gefunden. Die Angabe von ANDERS (1957, 1958), daß Histidin auch im Speichel der Reblaus auftritt, wurde von SCHÄLLER (1960) in Frage gestellt.

Im Zusammenhang mit der Ansicht, daß Spaltprodukte der pflanzlichen Nahrung über den Speichel wieder zurück in die Pflanze gelangen können und in Abhängigkeit von der Konzentration phytotoxisch wirksam werden, ist noch die Frage zu erörtern, ob bei Pflanzensaugern Regurgitation auftreten könnte. Ein Rücktransport von Nahrungssaft unmittelbar aus dem Darm in die Pflanze wäre der kürzeste und einfachste Weg, um die Spaltprodukte der Nahrung zur Wirkung kommen zu lassen. Trotz mehrfacher Untersuchungen (MILES, 1959; DAY & IRZYKIEWICZ, 1953; u.a.) gibt es jedoch keine Anhaltspunkte dafür, daß Regurgitation bei Pflanzensaugern regelmässig in Verbindung mit der Nahrungsaufnahme auftritt. Es muß deshalb auch weiterhin angenommen werden, daß das Speicheldrüsensekret eine Schlüsselstellung im Wechselspiel zwischen Wirtspflanze und saugendem Insekt einnimmt.

SUMMARY

AMINO ACIDS IN SALIVA AND HONEYDEW OF APHIS POMI *DEG., HOMOPTERA*

Recently it has been suggested that amino acids play an important part in the relationship between plant sucking insects and their host plants. A method was therefore developed to collect uncontaminated saliva from *Aphis pomi* and to estimate the amino acids in the saliva. To determine the accuracy of the method, the amino acids and amides on the honeydew produced by *Aphis pomi* were also estimated. Paper chromatography was used to estimate amides and amino acids.

The amino acids alanine, glutamic acid, aspartic acid, valine, serine and two ninhydrin positive compounds which have not yet been identified were found in the saliva of *Aphis pomi.* Alanine and glutamic acid occurred in the greatest amounts. In contrast, the honeydew of *Aphis pomi* contains 20 amino acids and the amides asparagin and glutamine in especially high concentrations. Threonine, serine and glutamic acid are also found in high concentrations. In analyses of the saliva, no asparagin, glutamine or threonine could be found, though these substances are present in high concentrations in the honeydew.

The significance of these facts is discussed. It is believed that amino acids in the saliva are breakdown products of the proteins in the food, and that they are circulated with the haemolymph to the salivary glands and then injected into the plant with the saliva.

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FURTHER STUDIES ON THE MAIZE AND RICE LEAF GALLS INDUCED BY *CICADULINA BIPUNCTELLA*

BY

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In the Philippines, plant galls were induced by the feeding of *Cicadulina bipunctella* (Mats.). Every insect collected in nature, as well as all insects raised on healthy maize, were able to produce leaf galls. Approximately 10 days after exposure to the insects, galls appeared on the next developing young leaf, but not on the leaf that was exposed to the insects. No difference was observed between the effect of feeding by single insects and groups of 10 insects. *C. bipunctella*-induced galls were compared to similar galls induced by the feeding of *C. zeae* in Africa, *C. pastusae* in South America, *C. bimaculata* in Australia, *Dalbulus elimatus* in America and *Euscelis plebejus* in Europe. In addition to experimental findings, worldwide association of leaf galls with feeding of leafhoppers belonging to several related species, favours the view that the galls of maize and rice plants are caused by insect toxins, rather than by plant viruses.

In 1924 OCFEMIA (personal communication) observed the occurrence of a gall disease of maize in the Philippines, but its etiology remained obscure for many years. AGATI & CALICA (1949, 1950) described the same leaf gall disease of rice and corn (maize) from the Philippines and linked it with the leafhopper vector *Cicadulina bipunctella* (Mats.).

Since then, a number of leafhopper-borne leaf gall diseases have been observed all over the world. H. G. GRYLLS & M. F. DAY (personal communication) in Australia found Wallaby Ear disease transmitted by *Cicadulina bimaculata* (Evans). In Africa, H. H. STOREY (pers. comm.) observed that *Cicadulina mbila* Naude and the related species *C. zeae* (China) cause the production of galls in maize plants. Storey observed over a number of years that *Cicadulina* spp. induce transitory vein swelling symptoms, followed by complete recovery; the appearance of these galls was irregular and there was no evidence found of a transmissible virus. The galls were found to be produced by a systemic factor, for insects feeding on the first leaf of a maize seedling would cause the vein swelling on the 3rd or 4th leaf. Storey also agreed that these symptoms closely resembled the Australian Wallaby Ear.

In Israel I. HARPAZ (pers. comm.) recently found a leafhopper vector *Calligypona marginata* (F.) (= *Delphacodes striatellus* Fallen) of the rough dwarf disease of maize (HARPAZ, MINZ & NITZANI, 1958). In Italy there occurs "*nanis-*

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mo ruvido", presumably the same disease as rough dwarf of Israel, but the Italian vector is still unknown (GRANZINI, 1958).

G. BRAVO & R. F. RUPPEL (pers. comm.) in Columbia (South America) associated vein swelling ("hinchazones") of wheat and barley with the feeding of *Cicadulina pastusae* (Ruppel & De Long).

Vein enlargements in clover were caused by the feeding of *Euscelis plebejus* (Zett.) in the Netherlands (MARAMOROSCH, 1953; DE FLUITER, EVENHUIS & VAN DER MEER, 1955). In 1959 an ephemeral disease of maize characterized by leaf galls similar to those of the Philippine disease was linked to the feeding of *Dalbulus elimatus* (De L. & W.) (MARAMOROSCH, 1959).

Cicadulina, Euscelis and *Dalbulus* are all related and belong to a group that is associated closely with grasses. *Calligypona* (*Delphacodes*) is not closely related to these leafhoppers.

Nature of the leaf galls

HARPAZ (1959) succeeded in the mechanical transmission of rough dwarf of maize plants using needle inoculations.

In 1955 DE FLUITER *et al.* described their tests with *Euscelis plebejus* and left the question open whether the clover disease discovered by MARAMOROSCH (1953) is of viral nature or whether it is caused by a toxin. Grylls & Day in Australia were unable to find a single *C. bimaculata* in all insects collected at more than 80 localities in Australia, and all of their progeny raised on healthy plants invariably transmitted Wallaby Ear. Since FUKUSHI's classical work on rice dwarf transmission through the egg of infective females (1933), a number of plant viruses were found to pass trans-ovarially to the progeny. However, a 100% "inheritance" of a virus would be very unusual indeed, and the inability to find virus-free insects eliminates the possibility of carrying out controlled tests.

Further studies on leaf galls in the Philippines

In 1960 an opportunity arose to renew studies on the leaf gall disease in the Philippines. Field collections of *Cicadulina bipunctella* were made on the island of Luzon. The insects were tested individually or in groups of from 10 to 25 insects on maize and rice plants. A stock colony of *C. bipunctella* was maintained on healthy maize plants and was used in parallel with the freshly collected insects.

It was found that all *C. bipunctella* collected in the field were able to induce leaf galls on maize and/or rice seedlings. The same was true of the presumably virus-free stock colony insects, although in most instances the maize plants in the cages, where the insect colony had been maintained, appeared healthy. This could be explained by the difference in the age of the maize plants: young seedlings, employed in the tests, were very susceptible, while older plants, on which the stock insects were breeding, showed no disease symptoms.

In a series of tests individual *C. bipunctella* were placed on marked leaves of 7-day-old maize seedlings, and transferred to fresh seedlings every 72 hours. In another series the insects were transferred every 24 hours.

In addition to individual insects, groups of 5 and 10 leafhoppers were also tested. The insects were confined to leaves by means of small cages attached by permanent magnets, as described by KALOOSTIAN (1955). Galls appeared in exposed plants after an average interval of 10 days. The vein enlargements did not, however, appear in the leaves that were fed upon, but on the next developing young leaf. Leaves formed subsequently remained free from galls, indicating the limited systemic movement of the gall-inducing principle. No evidence was found of further spread or multiplication of this principle in the plant.

While it was found that every individual insect was able to cause the disease, some insects induced galls on almost every day of feeding, while others did so irregularly. Longer feeding periods and larger numbers of insects per plant increased the probability of subsequent gall formation, as could be expected. Groups of 10 insects per plant induced galls very regularly. A few plants "escaped" infection, although 10 insects were placed on a leaf. Close examination showed that in such instances the magnetic attachment of the cage was at fault, and the insects were unable to reach the phloem of the leaf. When groups of 10 insects were caged for the same (24 hours) period on maize seedlings in conventional cages, with free access to all parts of the seedlings, leaf galls were always caused. No difference was observed between the effect of feeding by single insects or groups of insects.

DISCUSSION

AGATI & CALICA (1949) tried to transmit leaf galls by mechanical means and by dodder (*Cuscuta* sp.), but no transmission was obtained. The viral nature of the disease has been assumed on the basis of symptomatology, and of insect transmission tests. It appeared at the time that stock insects were virus-free, because plants on which they were maintained remained healthy. However, the difference in susceptibility of younger and older plants, discussed earlier, explains why older maize plants in the insectary could be considered "virus-free".

The inability to graft maize and rice plants and thus to carry out standard tests for virus transmission from plant to plant, together with the inability to transmit the galls mechanically or by previously "virus-free" insects, make it very difficult to prove that the disease is caused by a virus. The world-wide association of leaf galls with feeding of several related species of leafhoppers: *Cicadulina*, *Dalbulus*, *Euscelis*, and the failure to find *C. bipunctella* in the Philippines, or *C. bimaculata* in Australia that would *not* cause galls, strongly favour the view that the vein enlargings or leaf galls are caused by an insect toxin rather than a plant virus. Even if the galls could be induced by mechanical means, using the sap of plants or extracts of salivary glands, this by itself would not suffice to prove that the galls are of viral nature. Only if such induced galls would be further propagated in infected plants, that is, if the causative agent could be shown to multiply in infected plants, would convincing evidence be obtained against the toxin hypothesis. Also, if leafhoppers could be "cured" by heat treatment or other means and later "reinfected" by feeding on diseased plants, the question could be settled easily.

The described findings call for more experiments rather than for prolonged discussion. At present, most leaf gall diseases induced by leafhoppers are of minor economic importance. They pose, nevertheless, very interesting problems and should be studied more intensively in the future.

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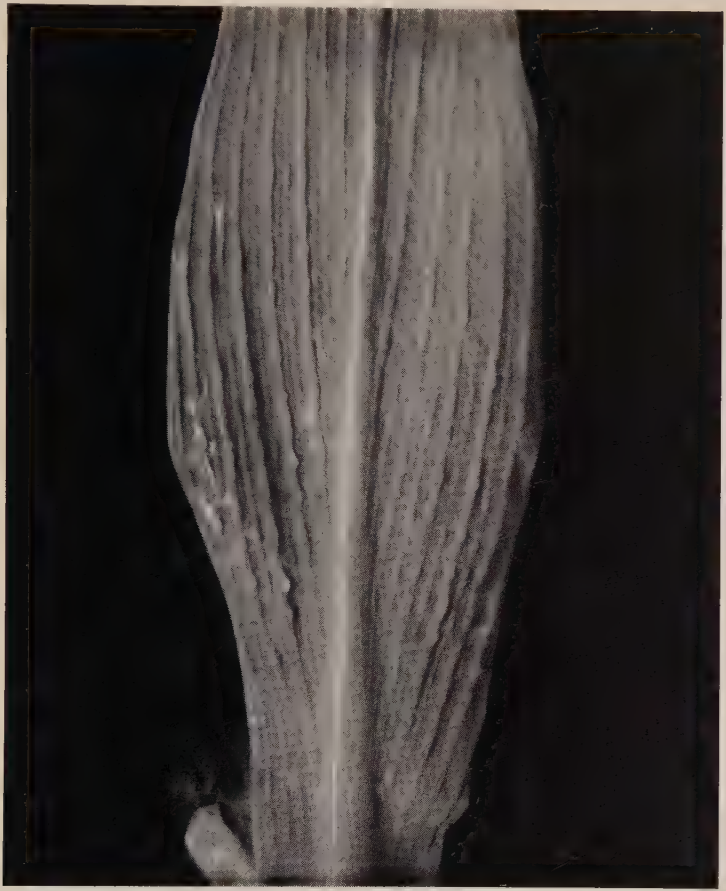

Fig. I. Leaf gall disease in maize, induced by feeding of *Cicadulina bipunctella.* Photograph by Jaime Nunez.

The authors are grateful to Dr. Robert F. Ruppel of the Agricultural Program of The Rockefeller Foundation, for an inspiring discussion and valuable information on leafhopper relationships.

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ZUSAMMENFASSUNG

WEITERE UNTERSUCHUNGEN ÜBER DIE VON CICADULINA BIPUNCTELLA *AN MAIS UND REIS HERVORGERUFENEN BLATTGALLEN*

Auf den Philippinen wurden Pflanzengallen durch das Saugen von *Cicadulina bipunctella* (Mats.) hervorgerufen. Jede dieser im Freien gesammelten Zwergzikaden sowie alle an gesundem Mais gezüchteten Individuen waren fähig, Blattgallen zu erzeugen. Ungefähr 10 Tage nach dem Ansetzen der Zikaden erschienen die Gallen auf dem nächsten sich entwickelnden jungen Blatt, jedoch nicht auf dem Blatt, das den Zikaden ausgesetzt worden war. Es wurde kein Unterschied zwischen der Wirkung des Saugens von einzelnen und Gruppen von 10 Zikaden beobachtet. Die von *C. bipunctella* hervorgerufenen Gallen wurden mit ähnlichen Gallen verglichen, die durch das Saugen von *C. zeae* in Afrika, *C. pastusae* in Südamerika, *C. bimaculata* in Australien, *Dalbulus elimatus* in Amerika und *Euscelis plebejus* in Europa erzeugt werden. In Ergänzung zu den experimentellen Befunden begünstigt die weltweite Verknüpfung von Blattgallen mit dem Saugen von Zwergzikaden, die zu einigen verwandten Arten gehören, die Ansicht, daß die Gallen an Mais- und Reispflanzen eher von Insektentoxinen als von Pflanzenviren hervorgerufen werden.

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ERRATUM

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pp. 192 and 193, Table VI.

Insert, correct or delete the following figures:

Larva No.	Days after 3rd moult	Insert	Correct	Delete
3	28	508		
4	28		508 to 466	
5	28			466
12	28			391
12	36		388 to 338	
13	17		369 to 360	

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ON THE BIOLOGY AND REPRODUCTIVE CAPACITY OF *SYRPHUS COROLLAE* Fab. (SYRPHIDAE) IN THE LABORATORY

BY

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Results of laboratory experiments on the biology of *Syrphus corollae* Fab. are presented. Characteristics of the reproductive capacity of the species are determined using demographic methods of analysis. Validity of results and their significance from the aspect of the biological control value of *S. corollae* are discussed.

The family *Syrphidae* represents an economically important group of insects since some of the species of this family are predators of aphids. But relatively little quantitative work has been done on these insects. In the following paper, results of laboratory investigations on one aphidophagous species of syrphid, *Syrphus corollae* Fab. are reported.

METHODS

A number of *S. corollae* females, which had been reared in the laboratory, were allowed to oviposit on leaves of pea plants on which aphids were feeding. After four hours, 298 eggs were collected. Larvae which hatched from these eggs were supplied with a surplus of aphids of the species *Macrosiphum pisi* (Kltb.) and *Aphis fabae* Scop. from a culture reared on peas. The larvae were kept in groups of 20 in petri-dishes covered by cloth gauze. Pupae were weighed on the day of pupation, numbered, and placed in petri-dishes in groups according to the date on which they pupated. Adults which emerged from the pupae were marked by clipping either the right or left wing or both wings and were then placed in large glass preserving jars. Clipping their wings seemed to have no influence on the vitality of adults. A layer of clear pebbles was placed in the bottom of each jar which was covered by cloth gauze and numbered.

Three males and three females were placed in each jar. The adults were fed hazel pollen and a solution of two volumes of water to one of honey. The pollen was presented on a filter paper in the bottom of each jar. Two pieces of absorbent cotton, one wetted in the honey solution and one wetted with water, were also added. A surplus of pollen was always present in each jar and the absorbent cotton plugs were frequently renewed with fresh honey solution and water. The jars were kept on shelves in a room in which daylight coming through the windows was supplemented by fluorescent lighting for 17 hours per day. Adults mated readily in these jars. The method thus far provided records of the length of larval and pupal stages, mortality in these stages, and adult longevity.

Every two days females were allowed to oviposit. Each fly was isolated for four hours in a small, wide-mouthed, glass jar covered with a close-fitting screen top. A pea leaf with aphids on it was placed in each jar. Aphids provide a qualitative stimulus for oviposition. Eggs laid by each female were counted and kept in separate petri-dishes. Records were kept of the fecundity of each female, the length of time required for embryonic development, the proportion of eggs which hatched, and the number of fertile eggs laid. Whether or not eggs of *S. corollae* are fertile can be determined by their colour on the day before they hatch. At this time, fertile eggs appear gray and may be slightly collapsed. Infertile eggs remain pearly white and either fully distended or completely collapsed.

Data were also examined for possible relationships between fecundity and pupal weight, between fertility of eggs and age of the mother, and between viability or proportion of eggs which hatched and age of the mother.

All experiments were conducted at temperatures between 22° and 24° C, relative humidities of 40% to 80%, and 17-hour photoperiod. It is emphasized that the experiments were carried out between the middle of February and the end of April since there are indications that the vitality of *S. corollae* may change at different times of year. Such variation in vitality or level of activity could be caused by changing photoperiod which was therefore standardized as much as possible in these experiments.

RESULTS AND DISCUSSION

Fecundity, Fertility, and Viability

The mean fecundity of 37 females, the percentage of their eggs which were fertile, and the percentage which were viable (proportion of the fertile eggs which hatched) are recorded in Table I. The value quoted for fecundity includes five females which laid no eggs at all. Fertility was based only on 16 females which laid fertile eggs. Viability was derived from consideration of the proportion of fertile eggs which hatched. Infertile eggs were not included since to consider them, in this case, would have given an erroneous impression of embryonic mortality as these eggs could not have developed under any circumstances. Fertility of eggs was less constant, on a day-to-day basis, than viability of eggs. Although some females never laid fertile eggs, others laid eggs of which a high percentage were fertile one day but on another day none were fertile. This phenomenon may have resulted because fertility is dependent to some extent on sensory stimulation of the ovipositing female. The particular conditions under which the ovipositing female finds herself may be influential in whether or not sperms are released from the spermatheca.

TABLE I

Mean fecundity, fertility (proportion of fertile eggs), and viability (proportion of fertile eggs which hatched) of S. corollae *at 22°—24° C, 40%—80% R.H., 17-hour photoperiod*

Mean fecundity	Mean % fertility	Mean % viability
403.9 ± 60.67	67.3 ± 6.90	69.1 ± 4.13

No significant relations were found between fertility or viability of eggs and age of females or between fecundity and pupal weight.

Developmental Time

Table II summarizes the lengths of egg, larval, and pupal stages. Time of embryonic development was relatively constant. All viable eggs hatched after three days of incubation. The length of larval and pupal stages were about the same; development of females was slightly longer than that of males in these stages. Average developmental time to emergence of adults was 18.2 days for males and 18.8 days for females.

TABLE II

Time of development of immature stages of S. corollae *at 22°—24° C, 40%—80% R.H., 17-hour photoperiod*

Length of egg-stage, days	Mean length of larval stage, days		Mean length of pupal stage, days	
	54 ♂♂	44 ♀♀	54 ♂♂	44 ♀♀
3	7.3 ± 0.17	7.7 ± 0.13	7.9 ± 0.05	8.1 ± 0.06

Sex ratio

Fifty-six adult males and 44 adult females emerged from the 298 eggs with which the experiment was begun. The sex ratio was 56 ♂♂: 44 ♀♀, or 1.3 ♂♂: 1 ♀. The total period of emergence of adults of both sexes was six days. The ratio, males to females, of the emerged adults on each of the six days is shown in Table III. Peak emergence of males occurred about a day before that of females. This was the result of the slightly faster development of males during larval and pupal stages.

TABLE III

Sex ratio at emergence of S. corollae *reared at 22°—24° C, 40%—80% R.H., 17-hour photoperiod.*

Day of emergence	1	2	3	4	5	6
Ratio, ♂♂ : ♀♀	5 : 0	33 : 17	15 : 22	2 : 2	1 : 2	0 : 1

Mortality

Table IV shows percentage mortality in the population in each of the immature stages. Infertility has been considered as mortality. Quoted pupal mortality is that of the F_1 population resulting from the original 298 eggs. Figures for embryonic mortality were derived from observations of eggs laid by the F_1 females. Larval mortality was estimated from observed embryonic and pupal mortalities in relation to total mortality in the immature stages.

Most of the mortality during immaturity occurred in the egg-stage. Infertility

TABLE IV

Mortality of immature stages in percentages of the original number of eggs of S. corollae *at 22°—24° C, 40%—80% R.H., 17-hour photoperiod*

Egg		Larva	Pupa	Total
Infertility	Inviability			
33	21	7	3	64

and inviability accounted for 84% of the total mortality (64% of the original number of eggs) up to the end of the pupal stage.

Certain adults, particularly males, which emerged from relatively small pupae were generally weak and died either during emergence or on the first day after emergence.

Longevity

Longevity of *S. corollae* adults is illustrated by survival rate in Fig. 1. The initial number of eggs was considered as the original total population. At the start of adult-life, the survival rates of males and females were the same. After the first few days of adult life, the proportion of surviving males was slightly higher than that of females, particularly as time progressed. Fifty percent of the adult males had died by the twenty-fourth day of adult life (survival rate = 0.16 of total original population of males), and 50% of the adult females were

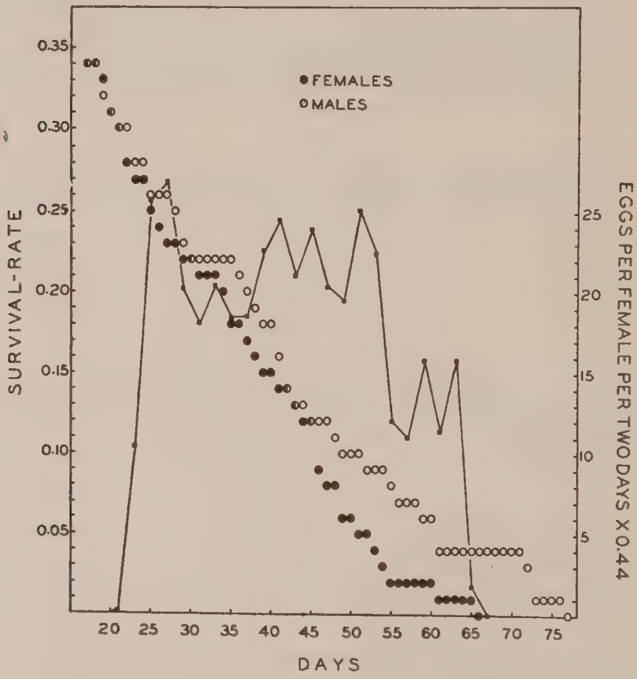

Fig. 1. Survival rate and age specific fecundity of *S. corollae* reared at 22°—24° C, 40%—80% R.H., 17-hour photoperiod.

dead by the twentieth day of adult life. The last male died after 60 days of adult life, or 77 days after the beginning of the experiment. The last female died on the fourty-ninth day of adult life which was the sixty-sixth day of the experiment.

Consideration of longevity as survival rate is more relevant to populations and their densities than simple statements about length of life, as will be emphasized in the following section. It is also a more meaningful method of stating longevity than mean values which might be misleading if the distribution of longevities was skew, as it usually is.

Survival rate and Age schedule of Fecundity

One of the basic factors in the efficiency of a predacious insect as a biological control agent is the rate at which populations of the predacious species are capable of increasing. There is little relation between the number of eggs, *per se*, laid by individuals of a species of animal and the abundance of that animal in nature, as ANDREWARTHA & BIRCH (1954) have emphasized. Natural abundance depends on the relationship between birth rate and death rate, or its inverse, survival rate.

Fig. 1 shows the survival rates of both sexes for the adult population of *S. corollae.* The solid line illustrates the age-schedule of fecundity which is the number of female eggs produced per female per two days. Since the female complement of a population is essentially its reproductive portion, only females were considered in these particular calculations. The total number of eggs laid in each two-day interval was therefore multiplied by 0.44, representing the sex ratio. This is the ratio which was observed for the F_1 generation, i.e., the adults which were produced from the original 298 eggs with which the experiment was begun.

Oviposition began on the twenty-first day of life of the population (taking birth as the date on which the eggs, which were to produce the adult-population, were laid). The highest rate of multiplication was reached about the twenty-sixth day (see Table V, $x = 6.5$).

The age schedule of fecundity reached a maximum early in adult life. This characteristic of the life cycle of an animal ensures a high rate of increase of that animal (BIRCH, 1948). It is not necessarily so, however, that the maximum rate of increase is always the most favourable rate for the survival of a species, since a balance must be maintained between reproduction of the species and the supply of food available. Food supply probably seldom becomes a limiting factor for aphidophagous species of syrphid flies, at least during summer months when aphid populations are increasing rapidly and pollen is readily available. It is true, of course, that reductions of aphid populations sometimes occur in mid-summer. Provided that the rate of increase of a syrphid does not, in fact, exhaust its food supply, the higher this rate, the more efficient becomes the species as a biological control of aphids. In this respect, graphs of survival rates and age specific fecundity rates can provide useful information in a comparison of the efficiencies of different species of syrphids as biological controls of aphids in a particular climatological area.

The observed rate of increase of any species of insect is determined by two different but inseparable categories of factors, (1) the physiological characteristics of the organism itself, i.e., the innate capacity of the animal for increase, and (2) the restrictions imposed by environment on this innate capacity. The innate capacity for increase of an animal depends upon its fecundity, longevity, and speed of development which, in a population, are reflected by birth rate and death rate. But a quantitative estimate of the rate at which a population increase or decreases is complicated by the fact that both the number of births and the probability of death change with the age of an animal.

LOTKA (1925) derived a function which he called "the intrinsic rate of natural increase" to take into account the variations in birth rate and death rate with age in human populations. Since then these methods have been applied to populations of mammals (LESLIE & RANSON, 1940) and insects of stored foods (BIRCH, 1948), (LESLIE & PARK, 1949), (HOWE, 1953a). The same methods have been applied here to the laboratory population of *S. corollae* to determine the net reproductive rate and the intrinsic and finite rates of increase, theoretical statistics which give a quantitative measure of the innate capacity of a species for increase and provide an index for comparison of different species as to their ecological valence.

The Net Reproductive Rate

The net reproductive rate is the multiplication of a population in one generation (LOTKA, 1945) and is referred to as R_0. The calculation of R_0 is based on the female population, the following data being required. (1) The life table for females which gives the probability at birth (oviposition) of being alive at age x (2) The age specific fecundity-table giving the mean number of female offspring produced in a unit of time by a female aged x.

The calculation of R_0 is shown in Table V. The age of females, listed in the first column of Table V is given in periods of four days. The four-day period facilitated the present calculation of the intrinsic rate of natural increase which requires the assumption that all eggs are laid at the mid-point of each age group. Since females were allowed to oviposit once every two days, each four-day period includes two periods of oviposition. The developmental period from the time of egg-laying to the middle of the first period of oviposition was 22 days or, in periods of four days, 5½ periods. The corresponding entry in the l_x-column indicates that only 36, or 28%, of the original 131 eggs which were destined to produce females survived to the midpoint of the first period of oviposition.

Column m_x is derived by dividing the total number of eggs laid during each period by the number of females alive at the mid-point of the period and multiplying this number by the sex ratio, in this case, 0.44.

The product $l_x m_x$ is obtained for each age group and the sum of these products is the value R_0. Here, $R_0 = 52.5$ which means that the population of *S. corollae* at 22°—24° C, 40%—80% R.H., and 17-hour photoperiod had the capacity to multiply 52.5 times in each generation. The worth of the statistic R_0 is limited because it must always be considered in relation to the length of a generation. Comparison of different populations, using their net reproductive rates as standards, may not be reliable unless the mean lengths of the generations are the same. However, R_0 gives an insight into the reproductive rate of a population under defined conditions.

TABLE V

Life table, fecundity rate, and net reproductive rate (Ro) of S. corollae ♀♀ *at 22°—24° C, 40%—80% R.H., 17-hour photoperiod. Sex ratio = 0.44.*

Pivotal age in units of four days	Life expectancy	Age specific fecundity rate	Reproductive rate
x	l_x	m_x	$l_x m_x$
5.5	0.28	10.3	2.88
6.5	0:24	51.5	12.36
7.5	0.22	37.1	8.16
8.5	0.20	38.2	7.64
9.5	0.16	39.8	6.37
10.5	0.14	44.4	6.22
11.5	0.09	48.5	4.37
12.5	0.06	41.5	2.49
13.5	0.03	37.1	1.11
14.5	0.02	32.3	0.65
15.5	0.01	27.3	0.27

$$R_0 = \Sigma\, l_x m_x = 52.5$$

The mean duration of a generation is defined as the mean period elapsing from birth of parents to birth of offspring. When T is the mean length of a generation, from the definition of net reproductive rate, $\frac{N_T}{N_0} = R_0$, where N_T = number at time T and N_0 = number at time zero, $R_0 = e^{rT}$ and $T = \frac{\log_e R_0}{r}$ where r is the intrinsic rate of natural increase. Using the value of r, calculated later in this paper, the mean length of a generation was $T = \frac{3.96081}{0.89} = 4.5$ weeks.

The Intrinsic and Finite Rates of Increase

A population in a non-limiting environment would increase according to the equation $\frac{dN}{dT} = rN$ or, in the integrated form, $N_t = N_0 e^{rT}$, where N_0 = number of animals at time zero, N_t = number of animals at time t, and r = the intrinsic rate of increase. The finite rate of increase (f), i.e., the rate of increase per female in a unit of time, is antilog$_e$ r from the relationship $\frac{N_{t+1}}{N_t} = e^r$, where N_t = the number of individuals at time t in a population increasing exponentially and N_{t+1} = the number of individuals one unit of time later.

The basic requirements for calculation of r are again those given in Table V. Howe (1953b) has presented simplifications of the method of calculation of r outlined by Birch (1948). One of these simplifications, the one used here, represents the observed pattern of oviposition by a number of eggs laid at a single instant, the mid-point of the oviposition period. If the developmental period of the species is *d* units and the period of oviposition is *l* units, all the eggs of the next generation are assumed to be laid after d + ½ l units.

If a female lays 2n eggs of which *p* mature and the sex ratio of offspring is 1, then she produces np mature female offspring. The selfmultiplicative rate of the species (*f*) is calculated as $\sqrt[d + \frac{1}{2} l]{np}$. The np values are those in the $l_x m_x$ column of Table V. The developmental period from the time of deposition of eggs to the middle of the first period of oviposition by the resulting adults was 22 days or, in periods of four days, 5.5 periods = (d + ½ l).

If eggs were laid only during the first four days of adult-life, the finite rate of increase ($f = \sqrt[d + \frac{1}{2} l]{0.44np}$) would be $\sqrt[5.5]{2.88}$ and log f = log $\frac{2.88}{5.5}$. Since $r = \log_e f$, then $r = \frac{(\log_e 2.88)}{5.5} = 0.19$. Because the total number of females in the population decreases with time, the number of eggs laid in a later period of adult life contributes less to the intrinsic rate of increase than a similar number laid at some period earlier (HOWE, 1953b), and therefore their effect is represented by a smaller number of offspring or eggs produced in the previous period. This can be seen from Table V.

Although the age specific fecundity rate at age x = 12.5 was 41.5 or about four times as great as that at age x = 5.5, the reproductive rate at age x = 12.5 was slightly less (2.49) than at age x = 5.5, (2.88), because there were fewer females left in the population at age x = 12.5. HOWE (1953b) has given a table of relative weighting coefficients by which the number of offspring of each oviposition period must be multiplied to transfer its effect to some other period, in this case, the first. Howe's table is partly reproduced here as Table VI.

Since the number of eggs laid after the peak of oviposition usually has little influence on the intrinsic rate of increase of a population, only the time to the end of peak of oviposition, the first seven periods, has been considered. The value of *r* for all 11 periods actually differs little from the value for seven periods.

TABLE VI

Proportional weights (expressed as percentage) attributable to the egg output of each of seven periods to calculate the weighted mean egg number per period (after HOWE, *1953b).*

r	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
Period 1	19	24	30	35	41	46	51	55
„ 2	17	20	22	24	25	25	25	25
„ 3	15	16	16	16	15	14	13	11
„ 4	14	13	12	11	9	8	6	5
„ 5	13	11	9	7	6	4	3	4
„ 6	11	9	7	5	3	2	2	
„ 7	10	7	5	3	2	1		

Using weighting values for *r* = 0.20, the equivalent number of eggs in the first oviposition-period of adult life for the first seven periods is: (2.9 × 24 + 12.4 × 20 + 8.2 × 16 + 7.6 × 13 + 6.4 × 11 + 6.2 × 9 + 4.4 × 7)/ 24 = 29.4. From the relationship, $5.5r = \log_e 29.4$, $r = 0.62$.

The procedure is now repeated using weights for $r = 0.62$, obtained by interpolation from Table VI, and the new value of r is 0.48. These calculations are repeated using successive values of r until r undergoes no further change. In this case the final value of r is 0.51 per period of four days or 0.89 per week.

The finite number of individuals arising from one female in four days is expressed by $f = \text{antilog}_e\ r = 1.67$, or 2.44 per week. Therefore, we estimate that a population of *S. corollae*, under the conditions of this experiment, has the capacity to multiply 1.67 times per female per four days. In eight days one individual will have produced $(1.67)^2 = 2.79$ individuals, and so on.

Analysis of the intrinsic rate of increase of a species of animal may provide useful information basic to studies of the same species in nature. Its distribution and abundance, the reason for its success in a certain environment or its efficiency, relative to other species, in performance of a certain function, such as parasitism or predation, may be explained by the particular innate capacity for increase of the animal or by the nature of one or more of the components (developmental time, fecundity, survival rate) of which it is a function.

With "natural" populations, it is necessary to consider certain checks on increase which are operative in nature but which were excluded from the experiments upon which the calculated values were based. Among these naturally operative checks on increase of syrphid populations are such things as: (1) access to pollen which provides a dietary factor necessary for oogenesis in females, (2) the presence of aphids which provide a qualitative stimulus for oviposition by syrphids, and the presence of aphids in sufficient quantity to support the larval population, (3) parasites and predators, (4) meteorological conditions.

Added to these natural potential hazards is the "unnatural" one of contact with chemical insecticides, to which *S. corollae* larvae seem particularly sensitive.

All these factors and certainly others tend to influence the actual rate of increase of a natural population of syrphids and make it more complicated than the theoretical r determined for a laboratory population. Despite this difference between the rate of increase of populations in nature and the intrinsic rate of increase calculated for laboratory populations, the statistic r does describe the maximum rate of increase that a population can attain over an indefinite period of time under defined conditions. Dependence of the intrinsic rate of increase on existing environmental conditions is actually an asset. It creates the possibility of comparing the innate capacities for increase of different species or the same species under different conditions. The intrinsic rate of increase provides an index for such a comparison.

Conclusions about populations in the field derived from results obtained by the present methods are subject to the inaccuracies already mentioned. They are, nevertheless, more reliable, in reference to field populations, than similar conclusions deduced from observations of fecundity, development, and mortality as separate entities. These conclusions are based not upon individuals, as such, but on their populations and it is with populations that we are necessarily chiefly concerned in most studies of numbers of insects in nature.

Experimental work involved in this study was done at the Institut für Pflanzenpathologie und Pflanzenschutz of the University of Göttingen. I am indebted to

Prof. Dr. W. H. Fuchs, Director of the Institute, and to his assistant, Dr. S. Bombosch, for their hospitality during that time. I wish also to thank Prof. Dr. D. J. Kuenen, Director of the Zoological Laboratory, University of Leiden, for his suggestions and helpful criticism of the manuscript.

ZUSAMMENFASSUNG

DIE BIOLOGIE UND DAS VERMEHRUNGSVERMÖGEN VON SYRPHUS COROLLAE *FAB. (SYRPHIDAE) IM LABORATORIUM*

1. Charakteristische Eigenschaften des Vermehrungsvermögens der Schwebfliege *Syrphus corollae* Fab. wurden für eine Kolonie bei 22° C — 24° C, 40%—80% r.F. und 17-stündiger Photoperiode festgestellt.
2. Die durchschnittliche Eizahl von 37 Weibchen betrug 403,9 ± 60,67. Am 21. Lebenstag begann die Eiablage (als Geburtsdatum wurde der Tag angenommen, an dem die Eier gelegt wurden, aus denen die Tiere schlüpften) und erreichte ihr Maximum am 27. Tag. Die grösste Vermehrung der Kolonie fand um den 26. Tag statt.
3. Die durchschnittliche Entwicklungszeit vom Tag der Eiablage bis zum Schlüpfen betrug 18,2 Tage für Männchen und 18,8 Tage für Weibchen.
4. Das Geschlechterverhältnis beim Schlüpfen war 1,3 Männchen zu 1 Weibchen.
5. Die Sterblichkeit während des Ei-, Larven- und Puppenstadiums betrug 66%, wovon die grösste im Eistadium festgestellt wurde. 50% der männlichen Tiere starben bis zum 24. Tag nach dem Schlüpfen, und 50% der weiblichen Tiere starben bis zum 20. Tag nach dem Schlüpfen.
6. Aus den vorhergehenden Daten wurde die Vermehrung der Tiere pro Generation mit 52,5 errechnet und die Vermehrung pro Weibchen pro Woche mit 2,44.
7. Die Bedeutung dieser Ergebnisse wurde in bezug auf *S. corollae* als ein biologisches Bekämpfungsmittel diskutiert.

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FAILURE TO REDUCE AN ISOLATED BLOWFLY POPULATION BY THE STERILE MALES METHOD

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In 1956 and 1957 pupae of *L. sericata* were irradiated at 6,000—7,000 rep, and allowed to emerge at three points on Holy Island. The sterilized population was maintained in preponderant numbers by regular replenishments. A test in 1958 showed no reduction in the density of the species. Possible causes of failure of the method are examined, and it is concluded that either the sterilization was inadequate or that the sterilized males were unable to compete with the native males.

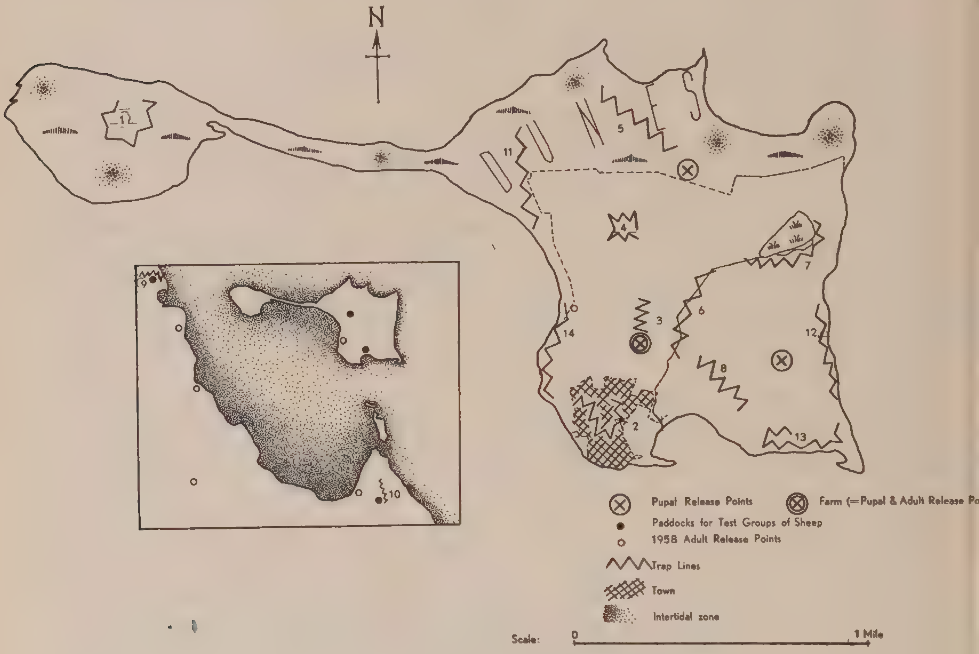
In 1955 American workers reported the elimination of the screw-worm fly, *Callitroga hominivorax* Cqrl., from the island of Curaçao in a single season, by the use of gamma-irradiated males (BAUMHOVER, GRAHAM, BITTER, HOPKINS, NEW, DUDLEY & BUSHLAND, 1955).

The principle of the method is most simply illustrated by numbers. Suppose that to a closed and stable population with 150,000 million females we add sterile but potent males in the proportion of say five to every one native male. Since a native female has only a one-in-six chance of fertile impregnation, the next generation should have 25,000 million females; the sterile population is maintained at its original level by replacements. The F_1 female population, reduced to one-sixth, has only a one-in-thirty-one chance of fertilization, so the F_2 generation will be only 1/186 of the original density, 806 million, the F_3 generation 806,000, and the F_4 1 female only.

The Sterile Males method is potentially a most important advance, in that its action is completely specific, and will remain so; the pressure on the survivors increases as their numbers decrease; it should continue to be effective, for it is difficult to imagine any species developing resistance. It is important therefore that the efficacy of the method be tested in the field against other species than the screw-worm; the blowfly (sheep maggot fly) *Lucilia sericata* Mg., because of its low density in nature (usually not more than 5/hectare), and because its field ecology was known in some detail, seemed suitable for a test.

TECHNICAL DETAILS

The results of preliminary work on the response of the species to gamma-irradiation have already been published (DONNELLY, 1960). They were: (1) A threshold somatic resistance to irradiation occurs when the pupa has completed about one-third of its developmental period. Older pupae survived irradiation doses of 24,000 rep. (2) Sterilization was practically complete at 6,000 rep. (3) Emergence was normal, at doses up to 12,000 rep, but longevity was progressively reduced with increasing dosage, and at 6,000 rep was approximately three-quarters that of normal flies. With pupae near the threshold age for survival at irradiation, the reduction of adult life was more severe than with older pupae. (4) Sterile males could inseminate females.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Pupal Release Points
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1958 Adult Release Points
Trap Lines
Town
Intertidal zone
Scale:
0
1 Mile

Fig. 1. Holy Island. Inset, adjacent mainland

For the test we selected Holy Island, situated two to five km offshore on the coast of Northumberland (Fig. 1). This island is approximately 5 km^2, and apart from a village at the southern extremity is agricultural country, mainly arable and grazing, with a northern strip of sand-dune and associated vegetation. We assumed, as a working estimate, a *L. sericata* population in the order of 750 flies per km^2, i.e. 2,000 males on the island. Our target was 5 : 1; with a four weeks longevity the sterile male population would require for maintenance a weekly addition of 2,500. With the pupal mortality estimated at one-third, an initial liberation of 30,000 flies was required and a weekly replacement of approximately 8,000. A method of rearing was designed, capable of yielding a continuous succession of fully fed larvae in sufficient numbers to meet this demand. It was arranged that our material be irradiated at Harwell, at a dosage of 6,000 rep, and at a minimum age of 5 days.

THE 1956 PROGRAMME

(1) Release and maintain throughout the season a 5 : 1 preponderance of sterile males.

(2) Make an initial estimate of the population density of *L. sericata* and other *Lucilia* spp. on the island. In order to distinguish subsequently between sterile and native *L. sericata*, released flies to be radioactive.

(3) Maintain two test groups of ten sheep each on the island, with two control groups at points on the mainland opposite the northwest and southeast corners of the island (Goswick and Ross). Once a week, for three weeks in every four, treat the sheep with ammonium carbonate to induce oviposition by *L. sericata*, as a measure of relative change in fly density. Initially, and every fourth week afterwards, make a series of bait-trappings and a release-recapture estimate of the populations of *Lucilia* spp.

The larvae were reared at outdoor temperature, pupated at room temperature, and were stored at room temperature prior to despatch for irradiation. The larvae were labelled with ^{32}P in feeding. The pupae were packed for irradiation, with a considerable bulk of peat, in cylindrical tins 20 cm high and 15 cm diameter. The first batch was returned to the laboratory, emerged there, and the adults brought to the island; the remaining batches were despatched direct to the island as pupae. Batches 1—6 represented our initial release. In Batch 7 onwards there were three tins per batch, one for each release point. The pupae and peat were placed in perforated metal containers sunk in the ground. After three weeks the residual material was brought back to the laboratory from one of the three release points in rotation, for an estimate of the emergence rate based on a count of dead pupae.

For the attractivation tests on sheep, a 4 cm × 1.5 cm cottonwool roll, soaked in 4% solution of ammonium carbonate, was sewn into the fleece over the loins. A small accessory plug, soaked in not more than 0.1% ethyl mercaptan, was sewn to the outside of the main plug. The plugs were put on the sheep in the morning, and removed in the evening along with any egg clusters observed. (Only *L. sericata* was bred from the eggs obtained). For the bait-trapping experiments minced liver, with 3% CaS stirred in, was used at the rate of 25 g. per trap. Twelve trap lines, each of ten traps, were staked out on the island, and one trap line at each mainland control area.

RESULTS IN 1956

The weather during the summer of 1956 was exceptionally unfavourable for blowfly trapping. Temperatures were low, and the rainfall quite abnormally high.

The target release of pupae was exceeded. Between 5 July and 14 July 32,000 pupae and over 5,000 adults were released. (Batches 1—6). By the end of September, over 170,000 pupae had been distributed on the island (see Table I).

There was an unexpectedly high and increasing pupal mortality as the season advanced; the emergence rate, initially 70—90%, was only 20—30% at the end of the season. This was at first attributed to the rain-sodden conditions, but examination of the returned samples in September showed that some at least of this mortality was intrinsic. The estimated nett release of adult flies on the island was about 90,000.

The schedule of tests was disrupted by the rain. Fifteen attractivation tests were made, in seven of which no oviposition was obtained even in the mainland controls. After mid-August no egg clusters were obtained on the island sheep in a series of five tests which yielded eggs on the control groups, but the last test of the season, on 21 September, yielded fertile *L. sericata* eggs on both Island groups of sheep.

Because of the poor trap catches it was not possible to make a reliable estimate of the local *L. sericata* population at the beginning of the experiment. An indirect estimate of the native *L. sericata* density may be made however from the release-recapture estimate of the related greenbottles, the *L. caesar* L. and *L. illustris* Mg. group (*L.c.* gp., see MACLEOD & DONNELLY, 1957). In the pre-release trappings the ratio *L.s.* : *L.c.* gp. was 1 : 7.

The *L. caesar* group was estimated at approximately 2,500 on the island (based on 5 recoveries only). On the basis of the above trapping ratio, the *L. sericata* population should in early July be 360 only. Alternatively, an estimate may be derived from the discrepancy between the estimate of total *L. sericata,* i.e. natives plus sterile releases, and the estimated number of surviving released flies. From the recoveries on days 2 and 3 after release, estimates of the total *L. sericata* population are approximately 10,500 and 11,000 (See Appendix, p. 118). Deducting approximately 5,000 survivors of the initial release, (plus an unknown but probably small number of teneral flies, the first emergences from Batch 2 pupae), we are left with < 6,000 native *L. sericata.*

The second attempt at a release-recapture estimate, at the beginning of August, was also unsuccessful; 150 baited traps caught, over three days, a total of only 150 *Lucilia* spp., including 44 *L. sericata.* Similarly, two later attempts in August yielded insufficient material.

In late September, in a final three-day trapping with 120 traps on the island and 40 traps on the mainland control areas, 38 *L. sericata* and 124 *L.c.* gp. were caught on the island, an approximate ratio of 1 : 3, whilst 12 *L. sericata* and 573 *L.c.* gp. were caught on the mainland, 1 : 48. From the first day's catch of *L.c.* gp. flies, 31 females were dye-marked and released, and 5 were recovered next day in a total female catch of 49; this gives an estimate of 300 females for the zone of influence of the traps, or under 2,000 females for the island. This is quite probable, as the *Lucilia* spp. adults are rapidly disappearing by late September, (MACLEOD & DONNELLY, 1957); the interesting point about it is that the total female *L. sericata* population, on a 1 : 3 ratio, would therefore be in the order of 700. That is, the continuing distribution of "replacement" pupae was not maintaining the population at anywhere near the target level. This failure is at least partly attributable to two causes: (a) the emergence rate of the later batches was very low (see Table I); (b) in nature, the *L. sericata* population, as judged by trap catches, disappears earlier than the *L.c.* gp. population (MACLEOD & DONNELLY, 1957).

In these various experiments the proportion of labelled flies in the *L. sericata* catches on the island rose from 7/44 at the beginning of August to 34/53 in late August, but was no higher (23/38) in late September.

In August some flies emerging from Batch 10 pupae were retained in the laboratory for several days. These flies were given a protein meal in their cages, and it was observed that they laid several egg clusters. As oviposition by these flies was contrary to expectation, a sample of 1,000 pupae was retained from each of Batches 14—17, and tested in the laboratory for fertility. In no case were eggs

TABLE I

Sterile flies liberated

	1956 (5 July—28 Sept.)			1957 (13 July—16 Sept.)			
		Emergence Estimates			Emergence Estimates		
Batch	Estimated No. of Pupae	Based on dead pupae		Estimated No. of Pupae	Based on dead pupae		Based on shells
		Estimated Emergence	% of Total		Estimated Emergence*	% of Total	% of Total
1	5,430 adults released			35,650	15,390	43	35†
2	16,500	17,120	92				
3	2,000			6,350	4,460	70	53
4	3,500	2,700	75	9,200	6,085	66	48
5	3,500	7,000	76	22,100	6,825	31	26
6	5,750			4,600	3,915	85	73
7	21,100	14,700	70	7,600	3,655	48	40
8	30,000	12,700	42	11,650	4,560	39	32
9	20,000	9,200	46	6,250	4,470	72	61
10	10,100	4,000	39	3,250	2,835	87	83
11	4,000	1,540	38	10,200	3,610	35	13
12	8,700	6,800	78	1,800	1,560	87	80
13	6,600	1,500	23	12,700	5,615	44	29
14	21,600	3,400	16	7,700	5,905	77	63
15	6,600	2,600	40		1,085 released as adults		
16	5,800	2,000		5,250	3,700	71	44
17	4,000			2,500	2,165	87	62
18				10,650	7,920	75	39††
19				8,650	7,340	85	50
20				11,100	6,170	56	59
Totals	174,750	85,500		177,200	97,265		

* Estimates of emergence are based on total pupae less laboratory samples.

† Batches 1 & 2, 1957, released as adults, less 1,801 died in cages.

†† 2,660 adults from Batch 18 not released on island.

obtained from sterilized flies. These irradiations, at 6,000 rep, revealed a low residual fertility in the males; a repeat test of the effect of different irradiation levels was therefore made, special attention being given at Harwell to rigid dosage control. The results confirmed the earlier findings, except that occasional fertile eggs were laid at male dosage levels up to 6,000 rep instead of 3,000 rep. In a further detailed test however complete sterility was obtained with 4,000 rep upwards.

Dosimetry tests on the possible variation within the container of peat and pupae were made for us at Harwell. It was found that in addition to the 5% fall-off from perimeter to centre due to the size of the container there was an additional 10% fall-off attributable to the density of the contents, (peat packing and pupae). In addition to this 15%, an inherent error of 8—10% in dosage was possible.

At the conclusion of the field work, a repeat test was made of the effect of irradiation and of radioactive labelling on longevity. The irradiated groups showed a reduction in longevity from the control (five weeks for 50% deaths) to two weeks. Labelling appears to reduce the expectation of life to 3—4 weeks, and also apparently reduced egg production and fertility.

THE 1957 EXPERIMENT

The following modifications from the 1956 programme were made.

1. The initial liberation target was raised to 20,000 males, with replenishments of 2,500 twice weekly.
2. The sterilizing dose was raised from 6,000 rep to 7,000 rep. Instead of three containers for each batch, a single cylindrical container, approximately 8 cm × 32 cm, was used, markedly reducing the proportion of peat to pupae.
3. A more critical control of the age of the pupae at irradiation was achieved by maintaining the pupae at 25° C instead of at variable room temperature.
4. A check sample of the pupae was extracted from each alternate batch on receipt at Holy Island, and returned to the laboratory for tests of sterility and adult longevity.
5. The emergence rate was estimated from the total residue of each batch, instead of from one-third.
6. The trays were redesigned as shallow vessels with a large emergence surface.
7. Radioactive labelling was discontinued, because of the apparent debilitating effect.
8. Progress during the season was estimated by twice-daily sheep-attractivation tests. The cottonwool plugs, put on in the morning, were examined at midday, and if negative were re-wetted with ammonium carbonate and mercaptan. Where egg clusters were present, they and the plugs were removed, and fresh plugs substituted. The sheep were again examined in the evening, and the plugs removed.
9. Twenty sheep instead of ten were used at each of the test points.

The test programme was as follows: (i) Bait-trap for one week in June to assess initial proportions of the different blowfly species. (ii) Do an initial release-recapture estimate of the *L. sericata* population. (iii) Maintain the sterile male population from July to mid-September. (iv) Distribute pupae at three points on the island, and bring back after three weeks the residual material of each batch, for assessment of emergence from counts of pupal shells and dead pupae. (v) Do attractivation tests on every suitable day, Tuesday to Friday, testing each egg cluster for fertility, and rearing all larvae for identification. (vi) For two successive days each month make a bait-trap survey of the blowfly species pattern on the island and control stations. (vii) Halfway through the season make a release-recapture estimate of the *L. sericata* numbers on the island, with comparative estimates on the control areas.

RESULTS IN 1957

Weather conditions were again adverse; of the three limiting weather factors for blowfly activity, viz., absence of sunshine when air temperatures are below 13° C, winds of over ten miles per hour, and steady rain, one or more was operative on most days from July to September.

The initial distribution of sterilized pupae began on 13 July with 37,500 pupae, along with 13,500 adults during the first week, and thereafter twice-weekly maintenance additions, until 21 September, by which time an estimated nett total of 93,000 adults had been liberated. The emergence rate of the pupae varied widely, but showed no tendency to the progressive decrease which occurred in 1956.

The initial bait-trap survey, at the end of June, gave the following *L. sericata*: *L.c.* gp. ratios, on the island (8 trap lines) and the mainland (4 lines):

Island,	80	traps,	39 : 63;	approximately	2 : 3
Goswick,	20	„ ,	16 : 84;	„	1 : 5
Ross,	20	„ ,	41 : 114;	„	1 : 3
Total Mainland	40	„ ,	57 : 202;	„	1 : 4

Results adequate for estimation of the *L. sericata* population were not available until late July, by which time irradiated pupae on the island had been emerging for some days. An estimate based on the discrepancy between the estimated total *L. sericata* on the island and the actual number of released flies gave 20,000 native *L. sericata* flies (see Appendix).

In mid-September a release-recapture estimate was made from a release of 1,750 dye-marked flies from Batch 18. On the first day of recovery trapping, 5 were recovered in a sample of 28 *L. sericata*, and, over the full week of recovery trapping, 7 in a total sample of 53. The estimates based on these are 10,000 and 12,000. The probable survivors, at this time, from the pupal releases can be calculated from Table I, taking weekly mortality as 25%, and emergence date as 3 to 4 days after pupal release.

Batch	Weeks since emergence		Survivors
14	3	(6,000/4)	1,500
15	2		500
16	1½	(3,700 × 37/100)	2,300
17	1	(2,165 × 3/4)	1,600
18	½	(7,920 × 7/8 — 2,660)	4,300
			10,200

The difference, — the native flies —, is on this basis in the order of 2,000. It is to be remembered that this was in mid-September, when the seasonal decline in adult density is well under way.

Attractivation results were enigmatic, because of the poor early and late results, so that the clear-cut difference obtained in mid-season, (during the only warm dry spell of over two days encountered), might be a stationary or increasing difference. The egg clusters per twenty sheep, with the number of "egg-blown" sheep in brackets, for the four fields were:

Period	*Island*			*Controls*		
	No. of Tests	Field 1	Field 2	No. of Tests	Goswick	Ross
10—26 July	9	6 (4)	17 (14)	6	8 (8)	8 (8)
31 July—2 Aug.	5	9 (8)	3 (3)	5	49 (37)	46 (42)
20 Aug.—28 Aug.	8	6 (5)	4 (4)	8	3 (3)	6 (6)
9 Sept.—4 Oct.	25	Nil	2 (2)	25	Nil	Nil

In all cases the egg clusters were fertile and the resulting adults identified as *L. sericata.*

1958 OBSERVATIONS

Since no clear-cut evidence of reduction of flies on the island had emerged during 1957, it was decided to test in 1958 for presence and relative density of *L. sericata* on the island. At the beginning of July a group of twenty sheep was put on the island, and a similar group on the Goswick-promontory. They were attractivated, using the 1957 method, at intervals during the next fortnight, and finally for three days at the end of the month. Weather conditions were quite good, but the total egg clusters obtained, over the ten days of test, were only 35 on the island (22 sheep blown) and only 4 (4 sheep) on Goswick.

Despite the at present inexplicable infrequency of oviposition on the mainland control sheep, there is clearly no evidence here of reduction of fly numbers on the island as a result of the experiment.

After the sheep tests were completed, a release-recapture estimate of *L. sericata* good, but the total egg clusters obtained, over the ten days of test, were released on the island, and trapping was continued over the following fortnight. 272 dye-marked flies were recovered along with 598 natives.

Three estimates may be made (see Appendix, p. 118):

(a) Assuming even distribution and no loss seawards: 3,800 flies released on 14 Aug.: death rate 1,000 per week; surviving flies on 19 Aug. (when the traps first cleared), 3,100; average available over 14—19 Aug., say 3,450; sample and recoveries on 19 Aug., 278 and 142. Estimate 6,700, less 3,100 released, say 3,500. This estimate is probably low, since the released flies may not have completely dispersed, i.e. there may have been more in the trapped area than in the remote parts.

(b) 25% mortality per week; survivors by mid-fortnight (= average numbers available during fortnight) 2,800; totals of daily samples and recoveries 870 and 272. Estimate: 9,000—2,800 or approximately 6,000.

(c) Estimate at release site on 19 Aug.: Survivors 3,100. Radius of zone of influence of traps 115 m, (circle slightly truncated by sea); zone say 0.04 km^2. Estimated availables = $115^2/183^2$ of 16%, i.e. approximately 6.5% of survivors, or 190. Sample and recaptures 83 and 59. Estimate = 267—190, or 77 for the zone of influence, i.e. 1,900 per km^2, or approximately 9,600 for the island.

The estimates of density per km^2 on the mainland, calculated from the recoveries at four release points, (see analysis of results), were 615, 635, 175 and 885.

ANALYSIS OF RESULTS

It would not have been a matter of surprise if we had failed to eradicate the species from Holy Island, because of its proximity to the mainland. The unexpected and important aspect of the result is the absence of any evidence of reduction of density of the species, after two successive seasons of application of the method. The theory of the method is unquestioned; the reason for failure must lie in faulty assumptions about the species, in the techniques, or in the conduct of the test. It is important that we find out whether the error was an avoidable point of detail or arose from a source inherent in the field application of the method. To this end, we shall review the possible explanations of our failure, and give some experimental evidence bearing on these.

Violation of the theoretical principles

The sterile : native ratio actually obtained in the field. The ratio, as assessed from the trap catches in 1956, varies as follows: 2—4.VIII, 7 : 37; 15—24.VIII, 34 : 19; 18—20.IX, 23 : 15. Though these very low catches are not necessarily representative, they do not suggest that the ratio exceeded 2 : 1 during 1956.

In the samples from Batches 14 onwards, the 50% longevity was about two weeks. The numbers of sterilized flies on the island at any date may be estimated from the Table I, by allowing an arbitrary period after distribution for completion of emergence. The two preliminary estimates of local *L. sericata* were approximately 350 and $< 6{,}000$. If we take 5,000 as the late July native density, and even allow a maximum delay of 2 weeks in emergence of steriles, the ratio to be expected would be 4.5 : 1 (23,000 : 5,000). By the time Batch 7 had emerged, the expected ratio, on the assumption of 5,000 natives, would be over 6 : 1, and would remain over 5 : 1 until early September, at which time the native *L. sericata* population would be expected to start showing seasonal decline.

The discrepancy between the estimated and observed ratios could be due to the following:

(a) Over-estimation of the field longevity of the sterilized flies. With regard to this, there is only this evidence: In 1957 the releases were not radioactive, but a special group of 1,000 labelled flies was released on 31st August. Specimens were recovered up to 25th September, i.e. about 4 weeks.

(b) Under-estimation of the native density. The initial density in 1956 may have been seriously under-estimated, but we do not consider this likely, from experience elsewhere (MACLEOD & DONNELLY, 1957, 1960), and in view of the 1958 estimate of 3,500—9,600 at the height of the fly season. We have no estimate for the beginning of the 1957 season. On the assumption of 5,000 native flies, the estimated sterile : native ratio during July and August was 5 : 1 or higher. That the initial density in 1957 was distinctly higher than in 1956 is however suggested by the enhanced *L.s.* : *L.c.* gp. ratio at the end of June, and by the estimate of 20,000 in late July. If this were so, the ratio might have been as low as 1 : 1, (for a 20,000 native population it varied from 1.2 : 1 to 1.6 : 1). It is conceivable that, even though the sources of error in 1956 had been reduced in the 1957 modified programme, such a low sterile : native ratio, applied over the season, might have failed to do more than bring an enhanced island population back to near its original level. We are still however left with the problem of what caused the increase in population in 1956.

(c) Loss of radioactive label. This was possible, particularly in the later stages of the experiment, through delay in pupation and in emergence. In monitoring checks on teneral adults, with 30- and 36-day old labels, 1 of 92 flies from Batch 14 gave a zero count, and 3 of 64 from Batch 16 gave counts of 20 or less. The error introduced by such loss could not however affect the main issue, — the actual ratio of sterile to native males on the island.

(d) Reduced response of treated flies to bait-traps. A small test was made in 1956 of the relative response to trap-bait of four groups of flies exposed as larvae or pupae to four treatments:

(a)	(Control)	85 released,	13	recovered.
(b)	(Radioactive labelling)	143 „ ,	6	„
(c)	(Irradiation)	150 „ ,	Nil	„
(d)	(Irradiation and labelling)	115 „ ,	Nil	„

A further test, with groups of 1,000 or more flies, was made in August 1957. Consistently wet weather resulted in a total recovery catch of only 26, so the experiment was repeated, with groups of 800 — 1,000, in October. The results of the two tests, expressed as recoveries per 1,000 viables released, are:

Radioactive:	Not Irradiated.	10.	50.	Irradiated.	5.	32.
Unlabelled :	„ „	11.	75.	„	0.	23.

These results suggest a seriously impaired responsiveness after irradiation; in the 1956 result there is the possibility of a similar effect due to radioactive labelling. The experiment needs repeating, because of a latent complicating factor not then realized, — the method of rearing (see later).

Multiple mating. It is generally held that, for the method to be effective, if a female mates more than once the sterile sperm must be able to compete successfully. A single insemination is sufficient to enable a *L. sericata* female to continue laying a succession of fertile egg clusters. Under cage conditions she may mate a second time, but she does so less readily, and there is no evidence that two matings can occur before oviposition.

The importance of multiple mating appears to us to depend on the type of behaviour implied by the term, and on the physiological mechanisms for fertilization in the species concerned (see also BUSHLAND, 1960). A. If the female copulates only once before each oviposition, then the effect, for the population, is the same as if the female were to mate only once in her life. B. If she accepts two or more inseminations before oviposition, the effect on the fertility rate should depend on whether the successive sperm loads remain "layered" in the spermatheca or mix. Where the sperm is passed directly into the spermatheca, as in the blowflies, if successive sperm layers do not mix, then the effect should be the same as for A. If mixing of the sperm of different origin occurs, then the efficiency of the method is reduced to an extent depending on whether or not the sterile sperm can fuse with the egg nucleus or otherwise obstruct fusion by another sperm. Thus: Assume a male sterile : fertile ratio of 5 : 1, and that six impregnations of each female occur before oviposition: (a) If the sterile sperm can compete on equal terms with normal sperm, then all the females may lay fertile egg clusters, but the proportion of fertile eggs in each cluster will be reduced on the average to one-sixth. The efficiency of the method will then depend on the reproductive potential of the species and the nature of the dominant factor(s) in the environmental resistance. (b) If the sterile sperm is unable to prevent fusion by a fertile sperm, then all the females will lay fertile clusters, with normal fertility rate per cluster.

Inefficiency of manipulative technique

The high level of the estimated *L. sericata* population, along with the relatively high *L.s. : L.c.* gp. ratio, early in the 1957 season, suggests that the *L. sericata* numbers had in fact been augmented by the 1956 releases. Since the species overwinters in the larval stage, this effect would imply that some of the releases were not sterile, or that in some way the addition of sterile flies to the population made more favourable the conditions for survival and/or reproduction of the fertile component of the population.

Inefficient sterilization. In the case of flies allowed to emerge and to mate under laboratory conditions, irradiation at 6,000 rep produced practically complete sterilization, (DONNELLY, 1960). After the disquieting observation in 1956 of oviposition by adults from one of the batches, it was found that a variation of up to 25% was possible with the technique being used, i.e. with a stated 6,000 rep the actual dosage might have been 4,500 rep, a level dangerously near the threshold.

The error was reduced in 1957 by substituting a different type of container, and by reducing very considerably the bulk of packing. The dosage was also increased to 7,000 rep. It is however possible that the change of container may have vitiated to some extent the advantage of uniformity of dosage by introducing another source of error: The much greater biomass in relation to the available air would cause a greater reduction in O_2 tension during the 40 hour confinement of the rail journey via Harwell to Holy Island. It is significant that the emergence rate of the 1957 batches was related inversely to size of batch, ($r = -0.82$; $P = < 0.001$). The reduced O_2 during the 18 hours prior to irradiation could have resulted in a reduced susceptibility to irradiation (see ALEXANDER, 1957).

There was no evidence of fertility in either sex when samples of pupae taken from alternate batches on arrival at Holy Island were tested, but this does not necessarily rule out the possibility that some of the emerging flies on the island may have had a higher degree of residual fertility than the negligible level anticipated.

Recovery of fertility. That recovery from irradiation sterility can occur seems now definitely established. The possibility of this having happened with *L. sericata,* because of anoxia at irradiation, (LUNING & HENRIKSSON, 1959), or because of delayed development of the pupae subsequent to irradiation, due to the low temperatures on the island, has yet to be tested.

Grossly reduced viability of the irradiated material. A reduction in viability greater than the expectation based on the preliminary tests could be due to two causes: departure from the test conditions in respect of the irradiation itself, and of the methods of production of the material for test.

In 1956 the pupal age-range at irradiation was 5—12 days, the minimum of 5 days being based on the "one-third of development" threshold for somatic resistance to irradiation. It was assumed that the upper limit of variation in the pupal period would not exceed 15 days. At distribution (6—13 days range), emergence had only occasionally begun. In Batch 10, estimated emergence rate 39, the actual emergence in the field was recorded. The possible age-range at emergence, for those pupae 5 days old at irradiation, was 12—17 days. That is, the youngest pupae of this batch may at irradiation have been on or dangerously near the threshold of (physiological) age for survival. In Batch 11, (estimated rate 38), emergence began on the fifth day after irradiation; assuming a 6 day spread of emergence, as occurred with Batch 10, this makes the age-range for the latest emerging flies 10—15 days, the pupae again approaching the permissible threshold at irradiation. In the case of these "threshold" pupae, the survivors would also have had a reduced expectation of life.

Lots of 92 and 65 flies, taken from the samples from Batches 14 and 15 returned to the laboratory for sterility test, were monitored as tenerals, in each case on the nineteenth day afer irradiation. Even allowing that this was the tail-end of the emergence curve, the age-range of the youngest pupae, at emergence, was probably 19—25. The estimated emergence rates for these batches are 16 and 40.

These estimates of age at emergence do not necessarily imply that the pupae were irradiated at or near the survival threshold of one-third development, as the delay in development may well have occurred mainly after irradiation, under the low temperature conditions at the release points. The possibility remains however that in 1956, though not in 1957, there was for this reason an appreciable mortality from the irradiations; some at least of the survivors would therefore have lives shorter than expected, and even their ability to fly and to mate may have been impaired.

An analysis of the effect of the mass-rearing method used is difficult, because of simultaneous variables. However, the possible rôles of the mass-rearing technique itself, of the radioactive labelling, of irradiation, and of the travel conditions experienced by the batches in reducing viability can be examined from the available records, and from some test results.

In 1956 the emergence rate of the successive batches of sterilized pupae, (including three final batches not released on Holy Island), dropped from the expected 70—80%, exceeded by the first seven batches, to $< 25\%$. The samples from Batch 14 onwards, tested in the laboratory for fertility, confirm the field estimates of reduced emergence rate, and show that the longevity also was reduced.

Batch No.		*14*	*15*	*16*	*17*	*19*	*20*	*21*
Emergence %	Sample	26(13)	57(15)	24(16)	21(16)	30(12)	25(15)	49(14)
(50% Longevity in brackets)	Lab.-normal Control	77(18)	64(30)			77 to 89 (?)		

All the batches, except No. 1, were radioactive; Batch 4 onwards were from larvae reared by the "continuous feeding stream" (C.F.) technique, as distinct from the routine insectary method of small isolated cultures used for lab.-normals. In the C.F. method, large egg-masses were added daily to the meat on a series of enamelled trays at cool room-temperature. These rested on a perforated metal shelf suspended over a floor of dry sand. Meat was added to the trays as required; the deserted residue of old meat was periodically removed. Fed larvae climbed out of the trays and dropped into the sand, from which they were collected twice weekly by sieving. The larvae were left in shallow aluminium boxes (about 300/box) at room temperature and the pupae removed weekly.

The larvae contributing to Batches 1—3 were reared by another method, — the Mass Feeder (M.F.), which also supplied the material used in the preliminary tests of the effects of irradiation. In this method a large mass of eggs was put with meat on a layer of sand in a metal bin at 25° C. Meat was added as required, but no further eggs, i.e. the culture was of a single age of larvae. The larvae pupated at 25° C.

In 1957 the larvae were obtained by the C.F. method, but pupated at 25° C.

The pupae were removed daily, and kept at 25° C or 30° C, so as to provide a batch of pupae of fairly uniform physiological age at date of dispatch to Harwell. They were not radioactive.

The analysis of the records is made in the following six paragraphs, numbered for later reference.

1. In the non-labelled M.F. material used for the preliminary tests and for the repeat test in 1957, the non-irradiated "travelled control" material showed emergence rates, as compared with lab.-normal material as follows, (bracketed figures = 50% longevity):

Emergence	85 (24)	89 (24)	90 (23)	97 (40)	80+ (24)
Controls	90	90	91	90	78 (35)

There is no evidence of any mortality effect from the mass-feeding technique or from the exigencies of travel.

2. In October 1956, four separate groups of pupae were sent to Harwell: C.F. pupae, radioactive, (1) for irradiation, (2) as travelled controls, i.e. differing only in respect of irradiation; M. F. pupae, not radioactive, (3) for irradiation, (4) as travelled-control. A non-travelled control sample of the C.F. material was also put up, while a contemporary lab.- normal culture provided a partial non-travelled control for the M.F. material. Emergence rate was noted, and groups of the resulting adults were tested for longevity (male/female).

C.F. radioactive label.	39 (14/10)	48 (26/25)	55 (21/21)
M.F. non-labelled.	74	69 (29/36)	88 (35/33)
	Irradiated	Travelled Control	Non-travelled Control

The figures suggest that: (a) the reduced longevity is associated with irradiation, but the pupal mortality doubtfully so; (b) neither the reduced longevity nor the pupal mortality is associated with travel, (the control group value 88 is not strictly comparable with the 69 group); (c) both effects are associated with either or both the C.F. rearing methods and radioactive labelling.

3. In two experiments in 1957 four groups of pupae, all of M.F. origin, were sent to Harwell, two being labelled, two non-labelled. One of each was irradiated. The material for these tests was reared as a special mass culture in an unheated outhouse; the conditions, other than the labelling of the larval food, were similar for the four lots. The first experiment was in late August, the second, almost identical, in mid-October. The emergence rates for the four groups and for contemporary non-travelled controls, in the two experiments, were:

M.F., labelled	84, 93	92, 95	87, 92
M.F., non-labelled	86, 93	76, 95	91, 90
	Irradiated	Travelled Control	Non-travelled Control

These results indicate that there was no debilitating effect due to labelling, to irradiation, or to the exigencies of travel.

4. Two experiments were made to test the effect of radioactive labelling on longevity: in the first, the male/female longevity was 19/28 as against 29/40 for the non-labelled control; in the second, 17/27 as against 28/40. In these, however, the controls were from a M.F. culture and a routine laboratory culture respectively, as the possible significance of the C.F. history of the test groups was not then appreciated.

5. For Batch 21 of 1956 a non-travelled control was set up; the emergence rate for this was 42, as compared with 49 for the irradiated material. This confirms 2 above, in that irradiation did not affect emergence rate and that pupal mortality in 1956 was not related to travel.

6. In 1957 the material for irradiation was not labelled. Samples were sent back to the laboratory, after travel, for test of viability and sterility; in some cases non-irradiated contemporary samples from the C.F. strain were put up as non-travelled controls. The comparative emergence rates for such pairs are:

Batch	9	11	13	14	20
Field sample	76	21	32	27	39
Non-travel control	50/71	0/69	76/88	86/89	76

(The double rates are for two collections of pupae, sampled before pooling to form the batch.) The debilitating effect is associated here with either irradiation or travel; it cannot therefore be entirely due to the C.F. rearing technique.

From the analyses of paras. 1 to 6 we come to the following conclusions: Irradiation, though reducing longevity, had no effect on emergence rate (2, 3, and 5) except that either irradiation or travel produced a reduction of emergence in the 1957 batches (para. 6). This could have been due to lack of aeration. The travel factor produced no effect (1, 2, 3, and 5), with the exception noted in 6.

The effect on both emergence and longevity associated with labelling (2, 4) may alternatively have been due to the C.F. method of culture. Labelling, by itself, had no effect (3); we conclude therefore that there was an irregularly occurring effect on both emergence and longevity arising from the C.F. method; that this effect was mainly responsible for the debility in the 1956 releases, and contributed. along with lack of aeration, to the less pronounced 1957 debility.

Specific unsuitability, for physiological reasons

Success of the Sterile Males method depends on the ability of the treated males to mate as readily as do normal males, and to produce in the female the same physiological responses, *e.g.* stimulation of egg production, maturation of the egg nucleus, and reduction of libido.

Preliminary observations on sterilized *L. sericata* indicated that morphologically normal sperm was formed, and that normal females could be impregnated by sterile males. This has now been confirmed. Because of the failure of the method in the field, a more detailed study of the physiology of mating and reproduction in normal and sterilized *L. sericata* is now under way. We hope to describe in a later paper certain experiments bearing on the question of mating adequacy and of sperm-layering.

Failure for ecological reasons

In August 1958 a test was made of the possibility of large-scale immigration from the adjacent mainland. Four groups of between 3,000 and 4,000 *L. sericata* were marked and released at points spaced over 6 km of the coastal strip of the mainland opposite the island. This coastal belt, for a depth of from 1 to 2 km, and the island, were trapped for the following fortnight, each with 200 traps.

Of the 15,800 flies released, 700 were recaptured on the mainland, but only 3 on the island. Since the mainland releases were caught at up to 5 km from their release points, it is clear that the material was adequately vigorous, and capable of dispersal; the 1.5—5 km wide tidal mudflats and sand separating the two areas appeared to be sufficient barrier to other than an occasional successful crossing.

In Fig. 1 the distribution of the trap-lines on the island is shown in relation to the farm, which was the release point for the release-recapture experiments in 1956 and 1957. In the July 1956 test, the recaptures on days 2 and 3, in relation to the total *L. sericata* caught at each trap-line, were:

Trap-line	1	2	3	4	5	6	7	8	11	12	13	14
Total caught	3	20	14	36	21	17	10	9	2	14	37	16
Recoveries %	Nil	25	21	21	33	11	10	11	Nil	29	16	19

Arranged in order of increasing distance from the farm, the recoveries values are: 21, 11, 11—19—25, 21, 0—10—29, 16, 33, 0; i.e. show no evidence of lack of dispersal.

In the July 1957 test, the relative incidence of recaptures was:

Trap-line	1	2	3	4	5	6	7	8
Total caught	4	62	29	25	21	38	5	20
Recoveries %	Nil	22	25	25	35	27	60	40

Apart from lines 1 and 7 where the totals were negligible there was no striking difference between areas in the incidence of recaptures in the catch. It seems justifiable to assume that the released flies dispersed over all the island, with the possible exception of the northwest prolongation of sand dunes.

DISCUSSION

We can conceive of only one set of circumstances under which failure to reduce the *L. sericata* population on Holy Island would be compatible with the proper functioning of the Sterile Males method, viz., where the native population density was at a ceiling level imposed by an environmental complex which operated only or mainly when this ceiling density was reached. Reduction of the viable egg output would then have no apparent effect until the declining population potential coincided with this ceiling.

Little is yet known of the relative significance of the limiting factors to natural increase of blowflies. Intra- and inter-specific competition between feeding larvae are known to be important for *L. sericata*, (SALT, 1932, ULLYETT, 1950, CRAGG, 1955), but the sources of possible pupal mortality have not been explored, nor is there any quantitative information on the loss from ground predation during the "walker" stage. Losses from insectivorous birds which chance on a concentration of teneral flies during peak emergence from a carcass site must frequently be high.

Assume for the moment that competition during the larval feeding period is the main limiting factor. In the absence of inter-specific competition a ceiling population in the order of 4,000 adults on the island could be replaced under normal conditions inside a month, by only 20 fertile females, each laying only one average-sized egg batch; if these females were distributed over the island, intraspecific competition would then be negligible, and the "ceiling" concept reasonable. But *L. sericata* forms only a small part of the blowfly population utilizing the same carrion. In early July 1956 the *L.s.* : *L.c.* gp.: *Calliphora* spp. ratio was 1 : 7 : 90, in late August 1 : 11 : 55, in August 1958 3 : 4 : 10. It follows that variation in the numbers of *L. sericata* larvae per unit carrion would normally have only a slight effect on the intensity of competition, i.e. that a normal island population of 4,000 *L. sericata* represents only the survivors of a very much greater larval population; the limiting factor here, competition in the carcass, would have operated with more or less equal severity on higher and lower *L. sericata* densities. It seems improbable therefore that the "ceiling" concept holds the explanation for absence of apparent effect.

If we accept that the failure is due to malfunctioning of the method, we are left with the following possibilities: it may have been common to both seasons, due to the same or different factors for the two, or it may have been confined to 1956, in which case a very considerable increase must be postulated, this increase of population largely absorbing the effect of the 1957 measures which would have been adequate for the pre-inflation population.

It seems that the method of rearing the material for sterilization resulted in considerable loss, which was greater and more consistent over the successive batches in 1956 than in 1957. In addition to the 1956 losses due to the rearing method there may have been some loss due to lack of aeration during travel, and also to pupal age at irradiation. In 1957 the rearing method seems to have had less harmful effect, but the aeration effect was apparently greater. The total reduction of available sterile males in either year does not however seem sufficient to account for the apparent absence of any positive effect on the native population. Similarly, the 1956 losses of sterile males would not cause any inflation of the overwintering population.

We are forced therefore to the tentative conclusion that the cause of failure lies either in (a) inadequacy of sterilization, in both years or in 1956 only, or (b) in inability of the sterilized males to prevent fertile insemination of the native females, this failure vitiating the effect of the releases in both years.

The conclusion underlines the warning note by KNIPLING (1960), who points out that excessive radiation damage, by affecting the competition between treated and normal males, may turn out to be the most significant obstacle to successful use of the method. There remains of course the possibility that chemical or other methods of sterilization may be perfected, but in the meantime it is clear that field application of the Sterile Males method must be preceded by an investigation not only of the mating behaviour of the sterilized male but of the effect of such mating on the female.

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ZUSAMMENFASSUNG

DAS VERSAGEN DER MÄNNCHEN-STERILISATIONSMETHODE BEI DER REDUKTION EINER ISOLIERTEN SCHMEISSFLIEGEN-POPULATION

Die Methode „sterile Männchen" zur Ausrottung einer Art zu benutzen, ist in ihrer Wirkung mathematisch zuverlässig, vorausgesetzt, daß die anerkannten biologischen Erfordernisse vorhanden sind; sie hat sich in der Praxis gegen *Callitroga hominivorax* (Myiasisfliege) als wirksam erwiesen. In einem in kleinen Maßstabe durchgeführten Versuch gegen die Goldfliege *Lucilia sericata* wurden Fliegen, die durch eine 6,000—7,000 rep Gamma-Strahlung sterilisiert worden waren, in überwiegenden Zahlen auf einer 5 km² großen Insel in zwei aufeinanderfolgenden Jahren freigelassen. Eine Reduktion der einheimischen *L. sericata*-Population ließ sich nicht nachweisen. Es wurde experimentell geprüft, daß eine Zuwanderung von Fliegen vom Festland zu vernachlässigen war; wiederholte Versuche bestätigten, daß die Bestrahlungsdosis ausreichte, um sexuelle Sterilität zu erzeugen. Der Ablauf und die technischen Einzelheiten der Versuche wurden analysiert in dem Bestreben, die Ursache des Versagens ausfindig zu machen.

Das Verhältnis steriler zu eingeborenen Männchen könnte im ersten Jahr infolge hoher Mortalität der sterilisierten Puppen under die erwartete Relation 5 : 1 gesunken sein. Diese Mortalität stand offenbar mit der Zuchtmethode in Beziehung. Es wurde jedoch gefolgert, daß dies an sich nicht für das Versagen verantwortlich gemacht werden könnte.

Er wird vorläufig angenommen, daß hier, als Ergebnis von Sauerstoffmangel, ein geringer aber signifikanter Grad von Resistenz gegen Bestrahlung vorliege, möglicherweise begleitet von einer ähnlich geringgradigen Erholungsfähigkeit von Strahlungswirkungen; andererseits versagte das sterilisierte Sperma in Konkurrenz mit fertilem Sperma bei Doppelbegattungen.

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APPENDIX

In estimating adult density by release-recapture, two methods were used:

I. Estimates from recoveries by a trap-line in the release area. (See 1958 estimates). The method (MACLEOD, 1958) is based on the assumptions that (1) traps distributed over an area of radius X metres sample an area of radius X + 90 m (X + 100 yds), — the "zone of influence" of the traps; (2) a released concentration of flies disperses so that after 3—4 days approximately 16% of the surviving flies are within the 10 hectares zone of influence of traps distributed over an area of radius 90 m (2.4 hectare) centred on the release point, and *pro rata* for traps distributed over smaller areas.

II. Estimates based on pooled recoveries from a number of trap-lines. A simplifying assumption is made here that the flies have dispersed evenly throughout the island — at least by the fourth day —, but not beyond the shores. The zone of influence of a trap-line is assumed to extend for 90 m from the traps. Traps were 23 m apart where possible, but frequently required to be closer. The length of a trap-line is taken as an average of 180 m, i.e. the zone of influence is 360 × 180 m. For 12 trap-lines this represents 0.9 km^2, or one-sixth of the island; for 8 trap-lines one-ninth.

1956. July estimates. Release 5,400. Survivors by 9 July 5,200.

Sample and recoveries 106 and 50. Estimate of total *L. sericata* on island 11,000.

10 July: estimate 5,000 × 146/69, = 10,500.

1957. July estimate. 9,800/2 females released 18 July.

Loss by Day 4, 15%. Survivors 4,150. 11% in zone of influence, i.e. 460.

Sample and recoveries on 22 July, (females only), 208 and 56.

Female estimate 1,700, i.e. 3,400 adults in zone of influence, = 30,600 on island.

This represents natives (+ a group of 3,000 unmarked adult releases) + Batch 3, (75% surviving), + Batch 4 (90% surviving).

Natives = 30,600 — 12,000 = approximately 19,000.

Mid-September estimate. Release 1,750 less 4%, 1,680. Sample and recoveries 28 and 5. Estimate 10,000. (This is almost certainly underestimated, since dispersal would be incomplete in one day.).

Mid-week estimate; average survivors = (1,750 + 1,300)/2, say 1,500. Estimate = 1,500 × 53/7 = 12,000.

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THE EFFECTS OF TEMPERATURE AND HUMIDITY ON THE REPRODUCTIVE POTENTIAL OF *APANTELES ANGALETI* MUESEBECK (BRACONIDAE: HYMENOPTERA)

BY

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Temperature and humidity are the two main factors that influence the rate of oviposition and the fecundity of *Apanteles angaleti* Muesebeck. At 90% relative humidity the same number of eggs is laid at 20, 25, 30 and 35°. Low humidities reduce oviposition.

The braconid *Apanteles angaleti* Muesebeck is a recently discovered larval endoparasite of the pink bollworm of cotton, *Pectinophora* (*Platyedra*) *gossypiella* Saunders. The pest has a world wide distribution and is a very important pest in all the cotton growing regions except Central America, Peru and Ecuador. The potential importance of the parasite can be seen from the fact that a very high percentage of the bollworms was found to be parasitised in the cotton field of the Indian Agricultural Research Institute and a large number of them have been imported by the United States Department of Agriculture for mass liberations and establishment in the Cotton growing tracts of the Southern and South-Western States. Attempts were made to mass breed the parasite in the parasite laboratory of the Indian Agricultural Research Institute and a technique has been evolved in 1953 that gets over main difficulties. This technique was further perfected in 1956 (NARAYANAN, SUBBA RAO & GANGRADE) and though conforming to the original description in principle, the new method described briefly below has been found to be more efficient and productive.

A number of species of the genus *Apanteles* is known to be difficult to breed in the laboratory. This initial difficulty having been overcome, investigations were made to study the parasite with special reference to its ecology to estimate the possibilities of the effective control of the pest in India and other parts of the world.

In this series of investigations, some studies were made on the rate of reproduction of the parasite at different temperatures and humidities. These data can be of value when planning liberation in a new area, and can give some information about the possible effect on the parasite in new surroundings. Of the many factors which influence the rate of reproduction, mainly temperature and relative humidity were studied. A few observations on light were also made.

MATERIAL AND METHOD

Corcyra cephalonica St. was used as an alternate host. The parasite was mass reared as follows:

Two glass troughs, 15 cm diameter and 10 cm depth, were obtained and a little water was put into one which was then covered with a piece of fine wire gauze. A thin piece of muslin was placed upon the wire gauze. A dish containing freshly hatched *Corcyra* larvae in a thin layer of flour was placed on the muslin cloth and the other trough was inverted over the first. Mated parasites were liberated in the upper trough for oviposition. The relative humidity in the upper chamber was about 90%. The parasites were fed with cut raisins. The dish with the exposed larvae was removed after 24 hours and replaced by a new dish with unparasitised *Corcyra* larvae. The parasitised larvae were transferred to large trays where plenty of crushed jovar (i.e. a type of *Sorghum*) with a little yeast was available for further growth and development. The *Corcyra* larvae completed their development and the parasite grubs emerged from the host larvae as the latter were just about to prepare their cocoons for pupation. The adult *Apanteles* in due course emerged from the trays.

Fifty *Corcyra* larvae (5 to 7 days old) were offered to a single mated female in a small Petri dish. The larvae were allowed to feed in a very thin layer of flour and the parasite was fed with cut raisins. Each experiment consisted of 3 replications. After 24 hours, the larvae were removed and a fresh batch of 50 was offered to the parasites. The parasitised larvae were dissected under a binocular microscope and the number of eggs laid was recorded. This procedure was continued until the death of the parasite.

The selected temperatures were obtained in incubators. Selected humidities were maintained in desiccators containing solutions of potassium hydroxide. The concentrations of the different solutions were taken from BUXTON (1931). The figures for relative humidity are only approximate and differ somewhat at the different temperatures, as has been shown by SOLOMON (1951) but are considered sufficiently accurate for this investigation.

OBSERVATIONS

Effect of temperature and relative humidity

The data recorded from daily dissections are given in Table I.

It will be seen that at all temperatures and at 90% relative humidity the same and the highest number of eggs was laid. Lower humidities always reduced the number of eggs. It is impossible to calculate exactly the interaction between temperature and humidity, because the survival time, and presumably the period over which the eggs was produced, differed under all combinations of these conditions. It would appear, however, that under hot dry conditions (25° C, 45 per cent relative humidity) the insects died before they could produce more than a very small number of eggs. Survival was, as might be expected, approximately inversely proportional to the saturation deficiency, except that at 35° C life was cut short. It is incidently rather surprising that the insects laid so well at such a high temperature which is unfavourable for reproduction in most insects, even those from tropical countries.

TABLE I

The effect of different temperatures and humidities on the egglaying of Apanteles angaleti.

Temp. °C	R.H.	Saturation deficiency min.	No. of eggs in each replicate R1	R2	R3	Total No. eggs	Average No. eggs	Average Longevity Days
20	45	9.6	46	45	37	128	43	5
	70	5.2	92	81	96	263	88	9
	90	1.8	131	108	112	351	117	9
25	45	13.1	45	49	65	159	53	4
	70	7.1	91	86	96	273	91	8
	90	2.4	125	111	133	369	123	9
30	45	17.5	39	38	37	114	38	3
	70	9.6	81	76	58	215	72	6
	90	3.2	128	107	132	367	122	7
35	45	23.2	20	17	9	46	15	2
	70	12.6	39	49	28	116	39	3
	90	4.2	142	116	90	348	116	4

Effect of light on the fecundity

The experiments were all conducted in the dark. Some experiments were conducted to find the effect of light and darkness on the fecundity of the parasite. Raisin was given as food. Table II shows the results of the experiments.

TABLE II

Showing the effect of light on the fecundity of Apanteles angaleti *R.H. 90%; Temperature 25°.*

	No. of eggs laid in each replicate			Average No. of eggs	Longevity of the female in days		
	R1	R2	R3		Min.	Max	Average
Light	125	141	127	131	9	10	9.3
Dark	120	129	102	117	6	10	9.0

The highest mean number of eggs laid is in light, the mean number being 131. This mean number is not statistically significant from those laid in darkness.

DISCUSSION

The parasite did not have a well defined pre-oviposition period and began ovipositing immediately after emergence. No change of feeding habits was seen before oviposition started. In fact the parasites were seen to oviposit readily even when they were denied of all food from emergence. AHMAD & GHULAM ULLAH (1941) observed in case of *Microbracon lefroyi* D. & G. that a low temperature of 16° C reduced the fecundity of the parasite. Increase of temperature upto 30° C also increased the egg production. At higher temperatures the number of eggs laid was reduced. Their studies on *Melcha nursei* Cam. (1945) showed that the highest number of eggs were laid at 16° C. In the course of the present investigations it was noticed if the humidity was favourable, the same number of eggs was laid at 20, 25, 30 or 35°. Identical observations were made by ULLYETT (1936) with *Microplectrum fuscipennis*; AHMAD & GHULAM ULLAH with *Microbracon lefroyi* D. & G.; and FINNEY, MARTIN & FLANDERS (1945) with *Macrocentrus ancylivorus* Rohwer. These findings are similar to that obtained in the course of the present investigations and it was found that at a higher level of 90% relative humidity the parasite lays the maximum number of eggs.

In concluding the discussion on the effects of temperature and humidity, presence and absence of light on the reproductive potential of *Apanteles angaleti*, it may be said that temperature and humidity, exert a profound influence.

We are grateful to Dr. E. S. Narayanan, Head of the Division of Entomology for his keen interest in these studies.

ZUSAMMENFASSUNG

DER EINFLUSS VON TEMPERATUR UND LUFTFEUCHTIGKEIT AUF DAS VERMEHRUNGSPOTENTIAL VON APANTELES ANGALETI *MUESEBECK (BRACONIDAE: HYMENOPTERA)*

Temperatur und Luftfeuchtigkeit sind die beiden Hauptfaktoren, welche die Eiablagerate und Fruchtbarkeit von *Apanteles angaleti* Muesebeck beeinflussen. Bei 90% relativer Luftfeuchtigkeit wird bei 20, 25, 30 und 35° C die gleiche Anzahl Eier abgelegt. Niedere Luftfeuchtigkeit verringert die Eiablage. Bei der Untersuchung des Lichteinflusses auf die Anzahl der produzierten Eier konnte kein signifikanter Unterschied gefunden werden.

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STUDIES ON THE RELATIONSHIP BETWEEN THREE SPECIES OF COLEOPTERA AND CERTAIN SPECIES OF ANNUAL MUSTARD AND RAPE

BY

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Experiments with annual mustards have confirmed American work which demonstrated that *Sinapis alba* L. and *Brassica nigra* Koch were less susceptible than *Brassica juncea* Coss. to infestation by larvae of the cabbage seed weevil (*Ceuthorhynchus assimilis* Payk.). The stem damage by cabbage stem weevil (*C. quadridens* Panz.) was less severe, and fewer stems were infested, on the variety of *S. alba* grown in the experiments, than on the other species. All the types of plant under observation were damaged by blossom beetles (*Meligethes aeneus* F.). Whilst it may be possible to select or breed a variety of *B. juncea* less susceptible to damage by the two weevils, it seems that the problem of blossom beetle damage is unlikely to be overcome by such methods.

KAUFMANN (1942) found that varieties of rape which flowered before blossom beetles (*Meligethes aeneus* F.) emerged in large numbers from hibernation and had a high proportion of large flower-buds by the time that the first beetles arrived, suffered less damage than plants which flowered later, and had many small buds when the beetles were numerous. STAPLEY (1948) also noted that turnip plants, which open all their flowers rapidly, were not so severely damaged as swede plants which have a few flowers open at once over a long period. In America DOUCETTE (1947) found that *Brassica juncea* Coss. was more severely infested by larvae of the cabbage seed weevil (*Ceuthorhynchus assimilis* Payk.) than either *Brassica nigra* Koch or *Sinapis alba* L.

In 1952 *B. juncea* var. Trowse replaced *B. nigra* as the crop grown in eastern England for the brown mustard seed used in condiment manufacture. The previous continental and American work led WINFIELD & GOUGH (1959) to believe that this change of species may have created new pest problems, and field experiments were carried out in 1958—59 to determine the relative damage by blossom beetles, seed weevils and cabbage stem weevils (*Ceuthorhynchus quadridens* Panz.) to several species of mustard and rape, with particular emphasis on the variety Trowse and closely related types.

EXPERIMENTAL METHODS

In 1958, there were two trial sites, one at Trumpington, Cambridge (Trial sown 27 March) and the second at Benwick, Isle of Ely, (Trial sown 16 April). The following six species were selected for trial: — i) Arlo turnip-rape (*Brassica campestris oleifera* var. *annua* Metz; ii) White mustard (*Sinapis alba* L.); iii) *B.*

pseudojuncea (A Svaløf cross between *B. nigra* and *B. campestris*); iv) *B. juncea* Coss. var. Trowse; v) *B. juncea* Coss. (Yellow seeded type). This species failed to germinate at Trumpington; vi) Black mustard (*B. nigra* Koch). In 1959 there was one site at Trumpington, Cambridge and on 16 March seed of the following six species was sown: — i, ii, iv, and vi as in 1958 plus vii) *B. juncea* Coss. (*nigra*-type similar to Trowse from central Russia), and viii) *B. juncea* Coss. (*campestris*-type leaf mustard from Japan).

The trials were laid out as 6 × 4 randomised blocks, each plot was 3.6 × 3.6 m, and the rows of plants were spaced 45 cm apart. A guard row of Trowse mustard was sown round each trial. Seed was sown at rates similar to those used commercially and in both years this resulted in plant stands of about eight per foot (30.5 cm) of row on the Arlo turnip-rape plots, and ten to fifteen plants per foot of row for the other species.

In 1959 Trowse mustard, *S. alba* and *B. nigra* were sown at fortnightly intervals, beginning on 18 March, in a preliminary attempt to assess the effect of sowing date on pest damage at Trumpington. At each sowing date there were two plots of each species, and each plot was 2 × 2 m with four rows of plants.

I have discussed in detail elsewhere the sampling methods for the adult insects, and for assessing their damage (Winfield, 1961). On the species trials at Trumpington regular counts were made of adult blossom beetles and seed weevils by shaking five plants per plot into a shallow tin tray. Blossom beetle damage was assessed by sub-samples of main racemes after petal fall; in 1958 five plants were taken from each plot for raceme sub-sampling and in 1959 twenty-five plants were taken, approximately halving the standard errors for the mean number of pods and blind stalks per raceme. In 1958 the standard errors expressed as percentages of their means varied from 9.52% to 13.76%, and in 1959 from 4.83% to 6.61% depending both upon species of plant and raceme position.

The infestation of pods by seed weevil larvae was estimated by samples of 100 pods per plot from the mixed Upper and Middle racemes in 1958; three separate 100 pod samples per plot were taken from Upper, Middle and Lower racemes in 1959.

The figures for damage and infestation by both blossom beetles and seed weevil larvae were accurate estimates but are comparative only and do not represent absolute losses of flower-buds or seed.

RESULTS

Meligethes aeneus

The beetles were much more numerous on the species trials than on field crops (Winfield, 1961), probably because of the relatively small area of the former. The number of beetles recorded on each species of plant at any given time seemed to depend on the weather and on the relative stages of development of the six species growing in the trial. In both years the population of beetles over the whole trial area reached a peak during the last fortnight in May and the first few days of June. The general build-up during May seemed to be closely related to the weather, and low temperatures delayed the infestation (see also Jary & Carpenter, 1948). Temperatures were a little lower than in 1958 during the

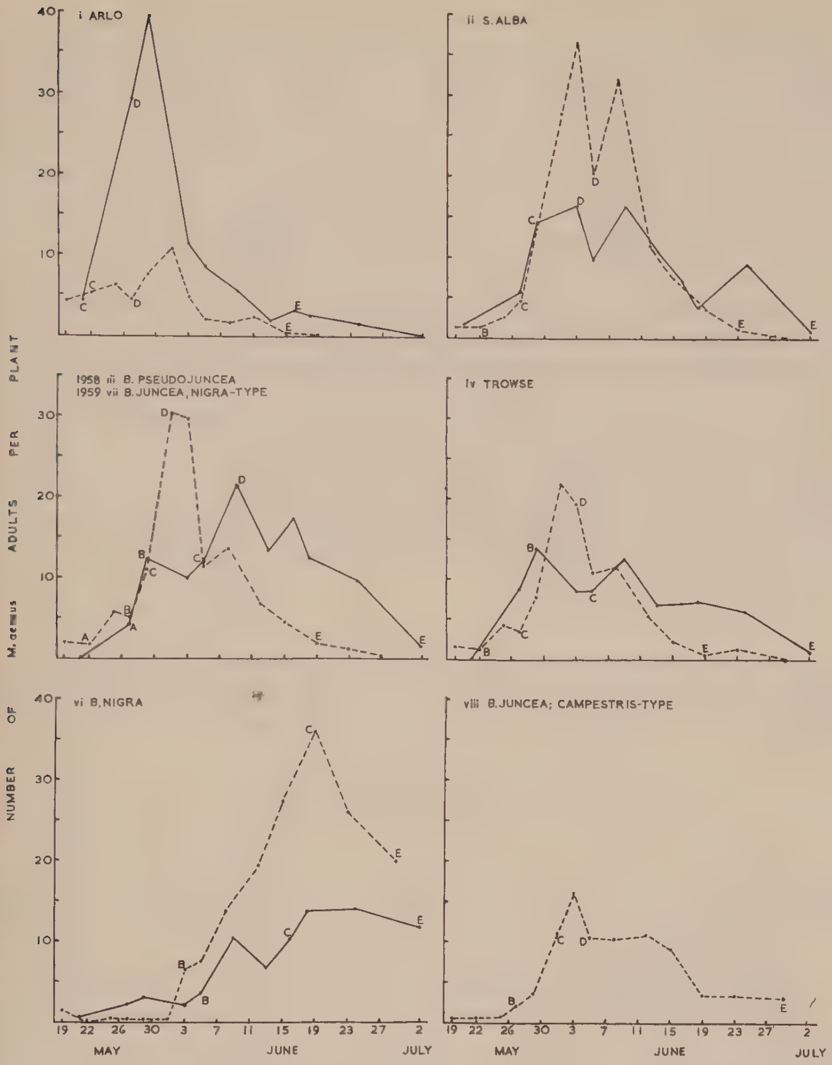

Figure 1. Population of adult *M. aeneus* at Trumpington 1958—1959. Unbroken line — 1958; Broken line — 1959. Plant stages: A 'Green-bud'; B 'Yellow-bud'; C 'First open flowers'; D 'First pods setting'; E 'Petal-fall'; Species numbered i—viii as in the section on methods.

last week of May 1959 and the build-up in beetle numbers was slightly delayed. Rainfall was generally much lower during the months of May and June 1959 than in 1958, although there was a showery period during early June in both years. These variations in weather conditions probably account for some of the fluctuations in the recorded beetle populations. Fig. 1 shows the population curves for each plant species, and letters are used to denote the stages of development of the plants (WINFIELD, 1961).

NOLTE (1959) discusses the attraction of *M. aeneus* to yellow coloured flowers and traps, and also to certain shades of green; WASSMANN (1926) and GÖRNITZ (1956) also describe experiments in which the beetles were strongly attracted by yellow colours. On the mustards the first beetles were found on the plants during the early green-bud stage, sometimes several weeks before any of the buds turned yellow, but the population did not begin to rise sharply until there were yellow buds on some of the older racemes. Arlo Turnip-rape, *S. alba* and the *juncea* group produced yellow buds and flowers over a period varying from 25—32 days. In 1958 (trial sown 27 March) there was a marked population peak on the Arlo turnip-rape plots which began flowering before the other species. In 1959 (trial sown 16 March) Arlo turnip-rape again flowered first, but the early build-up occurred on *S. alba* which was relatively more forward when the beetles arrived. This was probably because the whole trial was sown eleven days earlier than in 1958 and also because the cooler weather towards the end of May 1959 delayed the infestation of the Arlo turnip-rape. During late May and early June the population of beetles was fairly high on all the species except *B. nigra.* The yellow bud stage began in early June and lasted 11—16 days on *B. nigra* during which the population of beetles built up rapidly; all the flowers then opened more or less simultaneously, followed ten days later by almost complete petal fall.

TABLE I

Damage by M. aeneus *on the main species trials. Percentages of blind stalks (Angles). Means of UM and UML racemes, in 1958 and 1959 respectively. Species in order of flowering, Arlo turnip - rape first.*

Species	Trumpington		Benwick
	1958	1959	1958
Arlo turnip - rape	41.12	37.87	43.99
S. alba	36.01	38.89	32.86
B. pseudojuncea	53.81	—	45.59
B. juncea (*nigra*-type)	—	38.20	—
Trowse Mustard	59.91	41.08	50.36
B. juncea (Yellow-seed)	—	—	46.06
B. juncea (*campestris*-type)	—	45.60	—
B. nigra	44.92	45.98	46.75
Standard Error of the Treatment Means	± 1.441	± 1.071	± 1.732
Residual d.f.	12	15	15

The number of set pods, and blind stalks caused by *M. aeneus*, on the various raceme sub-samples are shown in Fig. 2, and Table I shows the percentages of blind stalks analysed by angular transformation. These percentages are averages for UM and UML racemes in 1958 and 1959 respectively. The two methods of showing the damage are given because larval damage was very severe in 1959, and percentages of blind stalks alone would be misleading. (See also WINFIELD, 1961).

Fig. 2 shows that there were less potential pods (blind stalks and set pods) on the middle and lower racemes than on the upper racemes of all the species,

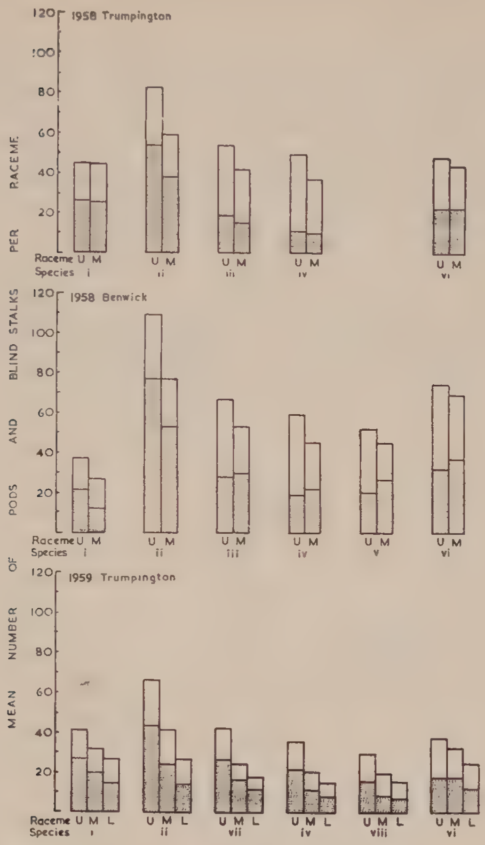

Figure 2. Comparative damage by *M. aeneus* to eight species of mustard and rape 1958—9. Each column represents the mean number of pods and blind stalks per raceme at UM and UML positions (WINFIELD 1961) in 1958 and 1959 respectively. The shaded areas are the numbers of set pods, and the unshaded areas the number of blind-stalks.

but that at all positions the *S. alba* racemes were the largest; in terms of percentage blind stalks therefore *S. alba* generally suffered less damage than the other species (Table I). In 1959, which was a very dry season, the larval damage was very pronounced, and shrivelling and browning of the raceme tips occurred during June on all the species (except Arlo turnip-rape). The tip-drop in 1959 resulted in lower counts of pods and blind stalks per raceme at petal fall (Fig. 2) and in Table I shows as similar figures for percentage blind stalks for all the six species.

From these observations it is clear that all the species in the trials suffered severe damage by *M. aeneus,* and that none of them produced large buds or open flowers sufficiently early to escape attack. *S. alba* and *B. juncea* had a flowering period long enough for many eggs to be laid in young flower buds, and for the larvae to become fully fed (MÜLLER 1941, SCHERNEY 1953).

Ceuthorhynchus assimilis

At maximum populations of both insects blossom beetles outnumbered seed weevils by 42 : 1 in 1958 and 32 : 1 in 1959. However, the population of weevils on each species closely followed the plants' development, reaching maximum at about the time the first pods were setting (Fig. 3). Open flowers probably have some attraction for the adult weevils as they were recorded even on species in which no larval infestation was subsequently found (Table II).

TABLE II

Percentage of Pods infested by seed weevil larvae, July.
1958 Average infestation in 100 pods from U and M racemes; 1959 average infestation on 3 × 100 pods from U, M and L racemes.

Species	Trumpington		Benwick
	1958	1959	1958
Arlo turnip - rape	5.6	41.9	12.0
S. alba	0	0	0
B. pseudojuncea	14.5	—	26.0
B. juncea (*nigra*-type)	—	20.5	—
Trowse Mustard	9.2	26.0	37.5
B. juncea (Yellow Seed)	—	—	22.5
B. juncea (*campestris*-type)	—	32.4	—
B. nigra	0.1	0.6	1.5
Standard Error of the Treatment means	± 1.90	± 2.58	± 4.52
Residual d.f.	12	15	15

Although some of the smaller differences are significant, the most obvious feature was the absence of attack on *S. alba* and the very low attack on *B. nigra*. This confirms the observations made in America by DOUCETTE (1947). Two slight attacks were recorded on white mustard field crops in 1959 (6% and 4% of the pods infested). Both crops were situated next to heavily infested Trowse crops, and the heaviest pod infestation of white mustard was found near the borders of the crops.

TABLE III

Damage on the sowing date experiment 1959 by M. aeneus *and* C. assimilis. *Two replicates of each plant species at each sowing date.*

Sowing date	*M. aeneus* Percent blind stalks (mean UML)			*C. assimilis* Percent infested pods (mean UML)		
	S. alba	Trowse	*B. nigra*	*S. alba*	Trowse	*B. nigra*
18 March	33.9	29.1	30.2	0	21.3	10.0
1 April	36.4	37.0	35.3	0	18.0	3.3
15 April	37.0	35.3	43.2	0	16.0	9.3
29 April	38.0	42.6	40.7	0	14.0	4.0
12 May	44.5	45.4	—	0	7.3	—

A sowing on 27 May failed because of the dry seed-bed

THE SOWING DATE EXPERIMENT 1959

The small trial at Trumpington gave interesting results, but the figures in Table III should be viewed with caution, as there were only two plots of each sowing date.

M. aeneus damage was worse on the later sowings mainly because there were fewer potential pods per raceme. On the Trowse mustard, for example, the average number of pods and blind stalks per raceme was 33.4 on the first sowing and 25.3, 24.1, 16.6 and 17.0 on the next four sowings.

On Trowse mustard the pod attack by seed weevil larvae seemed to be lower on the later than on the earlier sowings, which supports my previous observations on the effect of raceme age on pod attack (WINFIELD, 1961). On this trial also the *S. alba* plants suffered no infestation by seed weevil larvae.

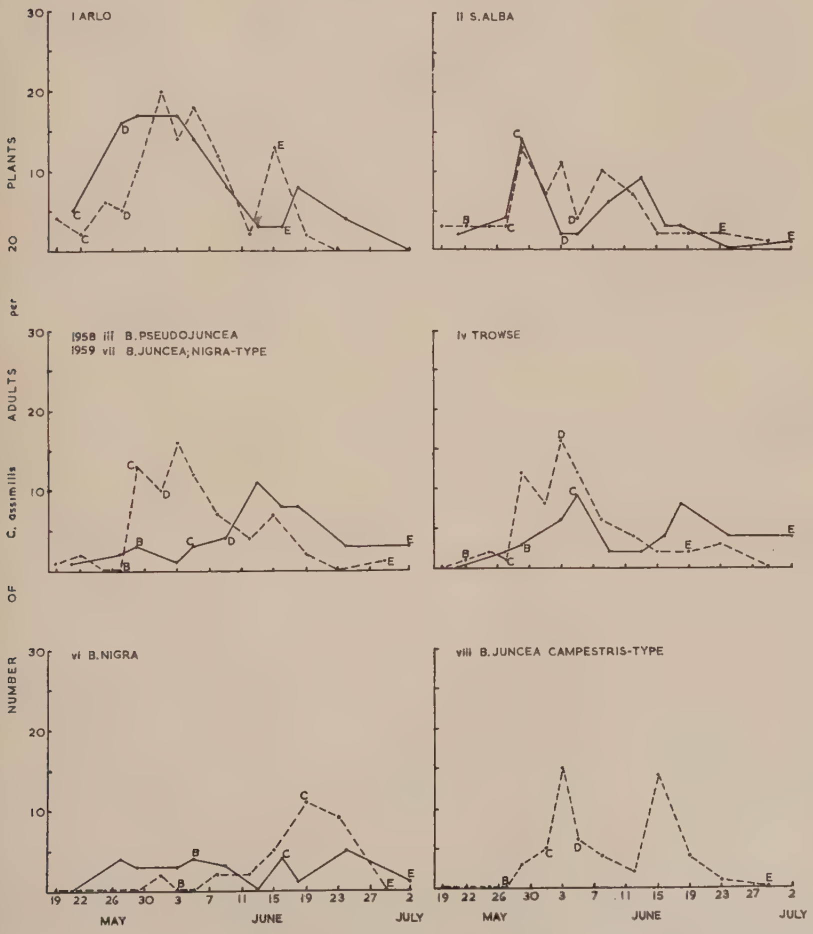

Figure 3. Population of adult *C. assimilis* at Trumpington 1958—9. Unbroken line — 1958; Broken line — 1959. Plant stage letters and species numbers as in Figure 1.

C. quadridens

Dmoch (1959) gives an extensive list of *Cruciferae* on which he and other workers have found *C. quadridens,* sometimes only the adults, but more frequently both adults and larvae. A number of stems of each of the species grown in the main trials were examined in the field for larvae and damage (Table IV).

Arlo turnip-rape was the most severely infested, and white mustard was only slightly attacked. For physical or physiological reasons the weevils may have refrained from laying eggs on white mustard, and the hollow dry pith of the variety grown in these trials may have been unsuitable for the larvae.

TABLE IV

Stem weevil larval attack on several species of Brassica *and* Sinapis.

Species	Stems infested			Trumpington 1959 % stems damaged Degree of damage		
	Max. 5		Max. 25			
	Trumpington 24.6.58	Benwick 8.7.58	Trumpington 25/29.6.59	Severe	Moderate	Slight
1) Arlo turnip-rape *B. campestris oleifera* var. *annua* Metz.	4.8	5.0	20.5	27	39	16
2) White mustard. *Sinapis alba* L.	1.5	1.0	4.8	1	8	10
3) *B. juncea* (a) var. Trowse	3.8	5.0	13.5	8	19	27
(b) *nigra*-type	—	—	13.0	5	21	26
(c) *campestris*-type	—	—	16.5	5	43	18
(d) Yellow-seeded	—	5.0	—	—	—	—
4) *B. pseudojuncea* (a Svaløf cross)	4.0	5.0	—	—	—	—
5) Black mustard. *B. nigra* Koch	3.5	4.8	13.0	3	23	26
Standard error of treatment means	± 0.44	± 0.10	± 2.10	—	—	—
Residual d.f.	12	15	15			

DISCUSSION

B. juncea has a long flowering period coinciding with the mass build-up in the populations of blossom beetles during May and early June, and the variety Trowse suffered severe damage by both the adults and larvae. *B. nigra* on the other hand has a short flowering period during late June and most of the damage to this species was probably caused by the adults. When *B. juncea* replaced *B. nigra* in 1952 the growers probably became aware of the damage by blossom beetles for the first time; *B. nigra* has many more flowers open at once than *B. juncea,* and it is possible that in the past blossom beetle damage had not been noticed. The field experiments showed however, that all the species including *B. nigra,* suffered severe blossom beetle damage, though it is possible that the effects of this insect were exaggerated by the small area of the trials. In the dry season of 1959 blossom beetle larvae caused more damage to the raceme tips than in 1958 which was cool and wet during June.

Continental observations suggested that the earlier flowering rapes escaped serious damage in some seasons, but the annual mustards tested in 1958—59 did not begin to produce buds and flowers until most of the adult blossom beetles had emerged from hibernation, and all species consequently suffered some damage. Although the number of buds destroyed on *S. alba* was the same or even greater than on Trowse and related varieties, the percentage damage was less because each raceme of *S. alba* is larger and produces many more buds.

Previous American work suggested that *B. juncea* was more susceptible than *B. nigra* or *S. alba* to attack by larvae of the cabbage seed weevil, and this has been confirmed during the present work. *S. alba* was also less severely attacked than other species by the cabbage stem weevil.

J. S. HEMINGWAY (personal communication) has noted differences in the degree of larval attack by both the weevils on a wide range of types within the species *B. juncea*, and in future it is possible that a less susceptible variety than Trowse may be bred or selected. It is improbable however, that any selection or breeding would produce a commercially acceptable variety of annual mustard which did not suffer some damage by blossom beetles.

I would like to thank Dr. H. C. Gough for his advice during the course of the work, and for his translation of some of the German papers; Mr. B. M. Church for his help with some of the statistical analyses; and Mr. E. A. Burton of Benwick for allowing me to carry out field trials on his farm in 1958. Grateful acknowledgement is also made to Miss J. A. Hewitt, Miss P. M. Yates and Mr. T. R. Williams for their assistance in the laboratory.

ZUSAMMENFASSUNG

UNTERSUCHUNGEN ÜBER DIE BEZIEHUNGEN ZWISCHEN DREI KÄFERARTEN UND GEWISSEN EINJÄHRIGEN SENF- UND RAPSARTEN

In den Jahren 1958/59 wurden Beobachtungen durchgeführt über den Schaden an den Blütenknospen verschiedener Senf- und Rapsarten durch Rapsglanzkäfer (*Meligethes aeneus* F.), über den Schotenbefall durch Larven des Kohlschotenrüßlers (*Ceuthorhynchus assimilis* Payk.) und über Befall und Schaden an den Stengeln durch die Larven des Gefleckten Kohltriebrüßlers *(C. quadridens* Panz.). In Feldversuchen wuchsen in mehrfacher Wiederholung folgende Pflanzenarten: *Brassica juncea* Coss var. Trowse und verwandte Arten, *Sinapis alba* L., *B. nigra* Koch und *B. campestris oleifera* var. *annua* Metz (Arlo turniprape).

Es wurde festgestellt, daß *S. alba* und *B. nigra* gegen den Befall mit Kohlschotenrüßlerlarven weniger anfällig sind als *B. juncea* und daß *S. alba* auch sehr viel weniger Stengelbefall mit Larven des Kohlschotenrüßlers erleidet als die anderen Arten. Alle geprüften Arten werden durch Rapsglanzkäfer geschädigt, doch mag die Schwere ihres Schadens durch die geringe Größe der Versuchsparzellen und die infolgedessen hohe Populationsdichte der Käfer pro Fläche und Pflanze übermässig vergrößert worden sein.

Es wird angeregt, eine geeignete Sorte von *B. juncea* mit niedriger Anfälligkeit gegenüber den beiden Käfern zu züchten oder zu selektieren. Es scheint jedoch, daß die lange Blütezeit von *B. juncea* und *S. alba* diese Arten besonders anfällig für den Befall mit Rapsglanzkäfern macht, und daß es unwahrscheinlich ist, daß Pflanzenzucht oder -selektion weniger anfällige Sorten hervorbringen können, die für die Handelsgewürzsamen-Anbauer wirtschaftlich annehmbar sind.

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ERRATUM

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Table I, p. 239. IV Instar "weight after" which reads 21.72, should be corrected to 217.2.

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DIE WIRKUNG EINIGER *SOLANUM*-ALKALOIDGLYKOSIDE AUF DEN KARTOFFELKÄFER, *LEPTINOTARSA DECEMLINEATA* SAY[1])

VON

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Die kristallisierten *Solanum*-Alkaloidglykoside (Solanin, Chaconin, Leptinin I, Leptinin II, Leptin I, Demissin und Tomatin) und ein amorphes Leptin III - Präparat wurden im Fraßtest an Kartoffelkäfern geprüft. Die Resistenz von *Solanum „chacoense"* gegen *Leptinotarsa* kann durch den Gehalt an Leptinen erklärt werden. Die Leptine gehen unter Abspaltung der Acetylgruppe in die Leptinine über und verlieren damit ihre hohe fraßabschreckende Wirksamkeit. DDT-resistente Käfer sind gegen *Solanum chacoense*, die Leptine und die Leptinine unempfindlicher als normale.

Von der Wildkartoffel *Solanum „chacoense"*[3]) selektierte TORKA (1949) gegen den Kartoffelkäfer und seine Larven homozygot resistente Pflanzen. Nach LANGENBUCH (1952) beruht die Resistenz auf einem Vergällungsstoff. KUHN & LÖW (1957, 1959 und 1961a, b) isolierten aus den Blättern dieser Pflanzen außer Solanin und Chaconin weitere Alkaloidglykoside, die sich von einem neuen Aglykon $C_{27}H_{43}NO_2$ ableiten und die sie Leptine nannten. Mit diesen Substanzen und mit den *Solanum*-Alkaloidglykosiden Chaconin, Solanin, Demissin und Tomatin wurden Fraßteste mit dem Käfer durchgeführt.

METHODE UND MATERIAL

Das Verhalten eines normalen und eines DDT-resistenten Stammes wurde gegenüber folgenden kristallisierten Substanzen geprüft: α-Solanin, α-Chaconin, Tomatin, Demissin, Leptin I, Leptinin I und Leptinin II. Ferner wurde ein amorphes Leptin III-Präparat (Präparat 24) geprüft.

Die normalen und die DDT-resistenten Tiere waren im Laboratorium aufgewachsen und stammten von etwa je 50 Gelegen verschiedener Weibchen. Die DDT-resistenten Tiere stammten von der Fa. Geigy, Basel. Sie waren kleiner und weniger pigmentiert als die normalen Tiere. Außerdem zeichneten sie sich durch eine besonders hohe Vermehrungsrate aus. Diese Eigenschaften blieben in unserer Zucht erhalten, sowohl bei der Selektion auf DDT als auch auf *Solanum chacoense*. Die Larven des normalen Stammes wurden auf *Solanum tuberosum* L. gezogen. Die Larven des DDT-resistenten Stammes wurden auf *Solanum tuberosum* gezogen, das kurz vor dem Füttern mit 0,1-prozentigem Gesarol gespritzt worden war.

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[1]) Herrn Professor Dr. RICHARD KUHN zum 60. Geburtstag gewidmet. Seine Frage nach dem Vergällungsstoff von *Solanum chacoense* führte zu diesen Untersuchungen.

[2]) Anschrift: Homburg/Saar, Institut für Physiologie.

[3]) Der Name *Solanum „chacoense"* ist strittig (= *Solanum subtilis* Bitt., vgl. BRÜCHER, 1954).

Nach dem Schlüpfen wurden die Käfer einzeln gehalten. Sobald ihr Fraß in vollem Umfang begonnen hatte, kamen sie 10 Tage lang in den Versuch, in dem ihnen abwechselnd 12 Std. lang eine Substanz zum Testen und 12 Std. lang wenig Futter (1 Scheibe *Solanum tuberosum*) angeboten wurden. In diesen 10 Tagen des „Reifefraßes" bestand keine Gefahr, daß die Prüfungen durch Unlust zum Fraß beeinträchtigt werden konnten.

Vor den Versuchen mit normalen Käfern wurde geprüft, ob die Blätter von *Solanum chacoense* gegen die Käfer resistent sind. *Solanum chacoense* wurde von den DDT-resistenten Käfern gefressen. Sie vermehrten sich darauf sogar, wenn auch etwa 95% der geschlüpften Larven sich nicht bis zur Imago entwickelten. Ein geringer Prozentsatz der normalen Käfer (unter 5% von über 1000 beobachteten Tieren) fraß auch an den Blättern von *Solanum chacoense*. Er wurde für die Teste nicht verwendet.

Die zu prüfenden Substanzen infiltrierten wir, soweit sie nicht wasserlöslich waren, als Hydrochlorid-Lösungen in 2—3 Wochen alte, frisch gepflückte Blätter von *Solanum tuberosum*. Die Bestimmung der infiltrierten Menge und der gefressenen Blattflächen geschah in der bei STÜRCKOW (1959) angegebenen Weise. Jedem Käfer wurde eine Blattscheibe 12 Std. lang angeboten. Von den Kontrollscheiben mit *Solanum tuberosum* hätte der Käfer in dieser Zeit 2—3 verzehrt.

Bei der Prüfung einer Substanz kam ein Käfer höchstens zweimal in den Test, bei der Prüfung verschiedener Substanzen aber wiederholte Male. Zu jeder Testserie gehörten eine Substanz bekannter Wirksamkeit, eine Kontrolle mit *Solanum tuberosum* und die zu prüfenden Stoffe. Während der Dauer der Versuche wurden die Käfer bei 25° C gehalten.

Die Abbildungen geben den Fraß in Prozenten an, bezogen auf den Fraß an der Kontrollscheibe, der gleich Hundert gesetzt wurde. Die ermittelten Konzentrationen infiltrierter Substanz (je kg frisches Blatt) pro Blattscheibe wurden zu Gruppen von 0,02% bzw. 0,2% vereinigt. Die Anzahl der Teste je Substanz und Konzentration ist in Tabelle I angegeben.

ERGEBNISSE

Frische Blätter von *Solanum chacoense* enthalten nach KUHN, LÖW & TRISCHMANN (1955) bis zu 0,35% Solanin und Chaconin im Verhältnis 1 : 1. Vorwiegend kommen α-Solanin und α-Chaconin vor. Die fraßabschreckende Wirkung von *Solanum chacoense* ist durch diese Glykoside nicht erklärbar (Abb. 1 und 2). Das Verhalten des DDT-resistenten Stammes ist bei Solanin und Chaconin dem Verhalten der normalen Käfer gleichartig.

Die in *Solanum chacoense* vorkommenden Leptine wirken als Vergällungsstoffe (Abb. 3 und 4). Leptin I stand in kristallisierter Form zur Verfügung. Präparat 24 enthielt weitgehend gereinigtes Leptin III. Der Gesamtgehalt an Leptinen pro kg frische Blätter beträgt mindestens 0,1%. KUHN & LÖW (1961a) vermuten, daß außer Leptin I und III noch die Leptine II und IV in *Solanum chacoense* enthalten sind. Leptin wirkte auf die normalen Käfer fraßabschreckender als auf die resistenten.

Die Leptine gehen unter Abspaltung des Acetylrestes in die Leptinine über (Tab. II). Dabei verschwindet die hohe fraßabschreckende Wirkung (Abb. 5 und

TABELLE I

Anzahl der Teste je Substanz und Konzentration der geprüften Alkaloidglykoside

Substanz	Konzentration in %	Anzahl der Teste		Abb.
		normale Käfer	DDT-resistente Käfer	
α-Solanin	0,2	11	7	1
	0,4	7	6	
	0,6	20	—	
	0,8	12	12	
α-Chaconin	0,08	11	—	2
	0,2	10	7	
	0,4	20	11	
	0,6	12	4	
	0,8	17	19	
Leptin I (kristall.)	0,1	6	—	3
	0,2	5	—	
	0,4	1	—	
Leptin III (amorph, Präp. 24)	0,01	5	—	4
	0,02	10	—	
	0,04	10	—	
	0,08	11	—	
	0,15	13	—	
	0,3	11	—	
	0,6	13	5	
Leptinin I (kristall.)	0,08	6	—	5
	0,2	16	—	
	0,4	14	—	
	0,6	11	—	
	0,8	12	11	
Leptinin II (kristall.)	0,4	11	—	6
	0,6	7	3	
	0,8	13	8	
Tomatin	0,01	7	—	7
	0,03	—	20	
	0,06	—	10	
	0,08	11	—	
	0,1	—	11	
	0,2	16	—	
	0,25	—	12	
	0,4	12	—	
	0,5	—	6	
	0,6	16	—	
	0,8	8	5	
Demissin	0,06	—	10	8
	0,08	16	—	
	0,2	13	15	
	0,4	16	6	
	0,6	10	—	
	0,8	15	7	

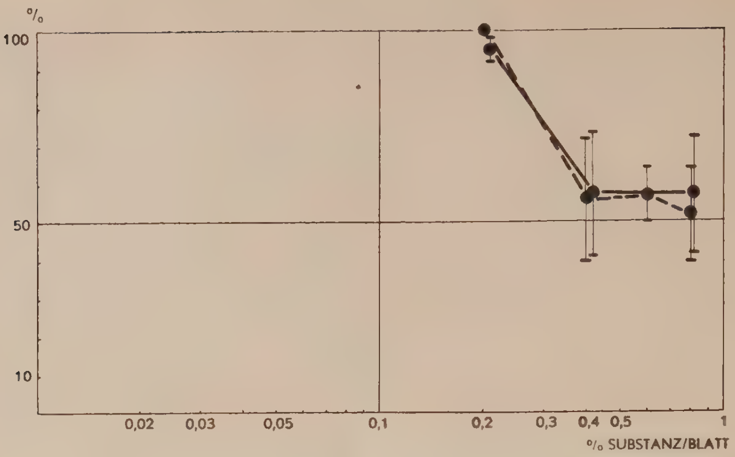


Abb. 1

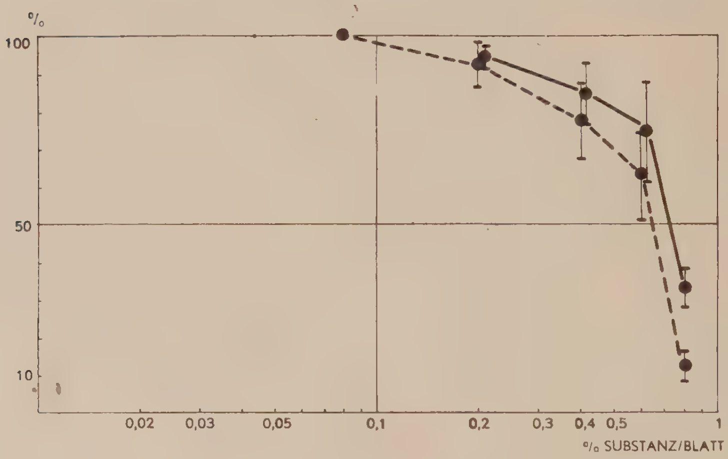


Abb. 2

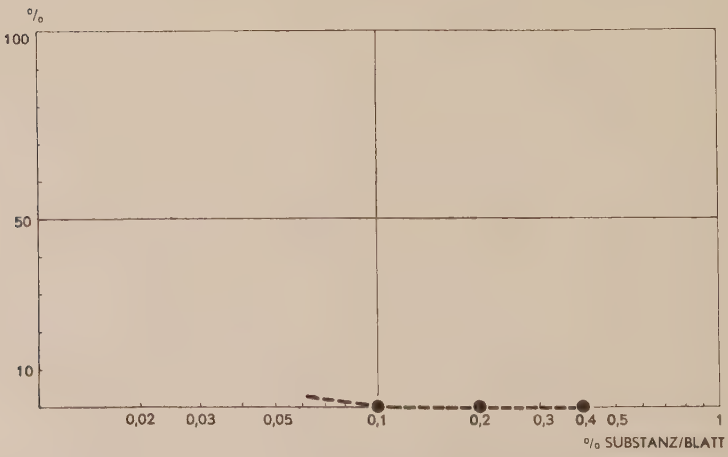


Abb.

Abb. 1. Teste mit α-Solanin. Abszisse: Konzentration Substanz je kg frisches Blatt. Ordinate % Fraß ± mittlerer Fehler. Gestrichelt: normaler Stamm. Ausgezogen: DDT-resistente Stamm. Abb. 2. Teste mit α-Chaconin. Gestrichelt: normaler Stamm. Ausgezogen: DDT resistenter Stamm. Abb. 3. Teste mit kristallisiertem Leptin I, normaler Stamm.

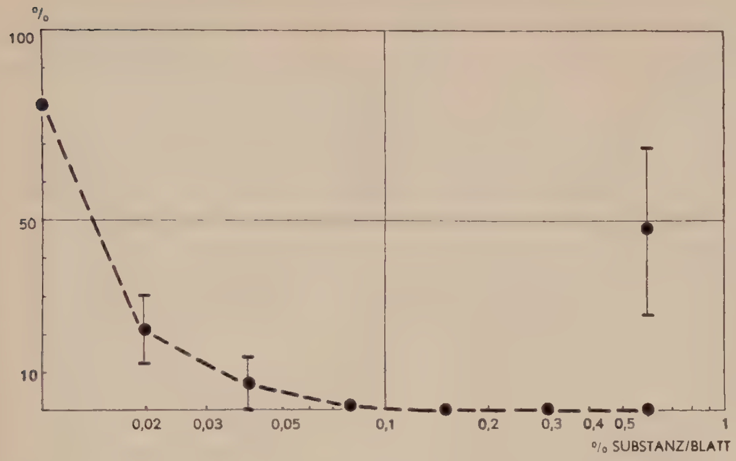


Abb. 4

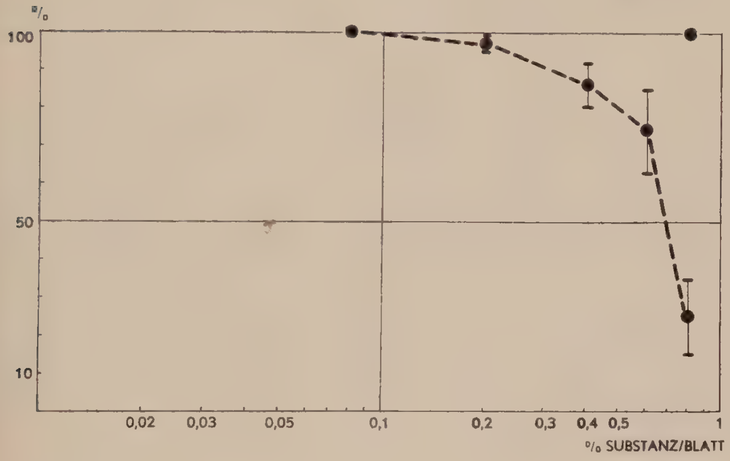


Abb. 5

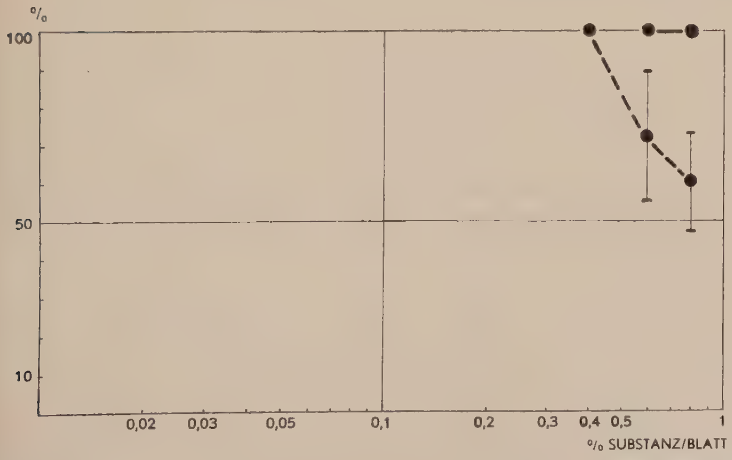


Abb. 6

Abb. 4. Teste mit Präp. 24, amorphes Leptin III. Gestrichelt: normaler Stamm. Einzelner Punkt: DDT-resistenter Stamm. Abb. 5. Teste mit kristallisiertem Leptinin I. Gestrichelt: normaler Stamm. Einzelner Punkt: DDT-resistenter Stamm. Abb. 6. Teste mit kristallisiertem Leptinin II. Gestrichelt: normaler Stamm. Ausgezogen: DDT-resistenter Stamm.

6). Die Leptinine wirkten auf die normalen Käfer auch fraßabschreckender als auf die Resistenten.

TABELLE II

Aufbau der kristallisierten Alkaloidglykoside

Alkaloidglykosid	Aglykon	Zuckerrest				
Chaconin	Solanidin	Gl	Rh	Rh		—
Leptin I	Leptinidin	Gl	Rh	Rh		Ac
Leptinin I	Leptinidin	Gl	Rh	Rh		—
Solanin	Solanidin	Ga	Gl	Rh		—
Leptinin II	Leptinidin	Ga	Gl	Rh		—
Demissin	Demissidin	Ga	Gl	Gl	Xy	—
Tomatin	Tomatidin	Ga	Gl	Gl	Xy	—

Gl = Glucose, Ga = Galaktose, Rh = Rhamnose, Xy = Xylose, Ac = Acetylgruppe

Tomatin und Demissin wirkten dagegen auf die normalen Tiere weniger fraßabschreckend als auf die resistenten (Abb. 7 und 8).

In Abb. 9 sind die Prüfungen mit den verschiedenen Alkaloidglykosiden zusammengestellt. Die vergällende Wirkung von Tomatin und Demissin ist wesentlich geringer als die der Leptine. Außerdem ist sie anderer Art. Auch bei hohen Konzentrationen fressen alle Käfer noch ein wenig von den Scheiben. Bei Leptin wird entweder gar nichts gefressen (es werden zwar probierende Einbisse ohne nachfolgenden Fraß gemacht) oder es wird gleich eine größere Menge gefressen. Aus den Häufigkeiten des Fraßes im Vergleich mit den gefressenen Mengen ist dieses zu ersehen. In Tab. III sind die Zahlen fressender und nicht fressender Käfer aus allen Prüfungen zusammengestellt.

TABELLE III

Häufigkeiten des Fraßes von den Prüfungen in Abb. 1—8

Substanz	normaler Stamm fressende: nicht fressende Käfer	DDT-resistenter Stamm fressende: nicht fressende Käfe
Tomatin	68 : 2	63 : 1
Demissin	70 : 0	38 : 0
α-Chaconin	64 : 6	40 : 1
α-Solanin	50 . 0	25 : 0
Leptinin I	57 : 2	11 : 0
Leptinin II	27 : 4	11 : 0
Leptin I	0 : 12	nicht geprüft
Präp. 24 (Leptin III)	11 : 62	4 : 1

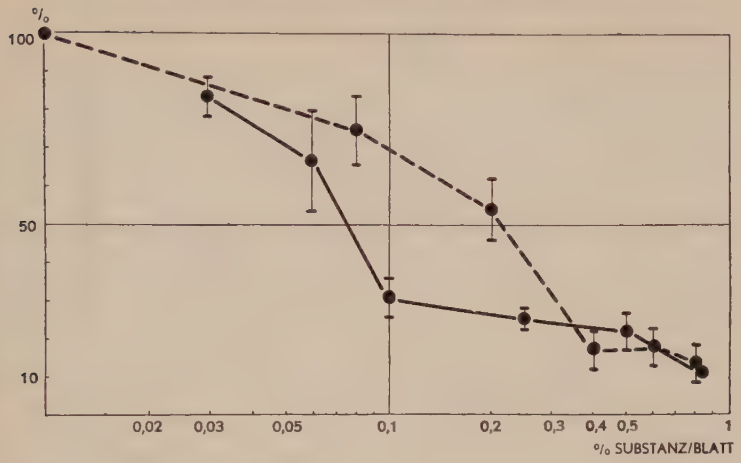


Abb. 7

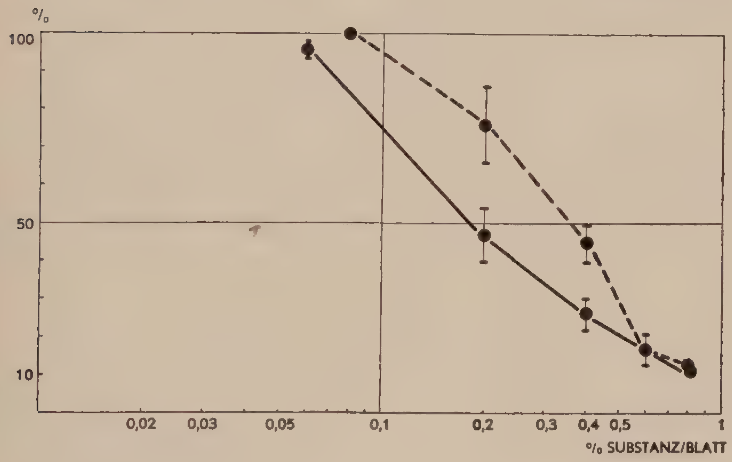


Abb. 8

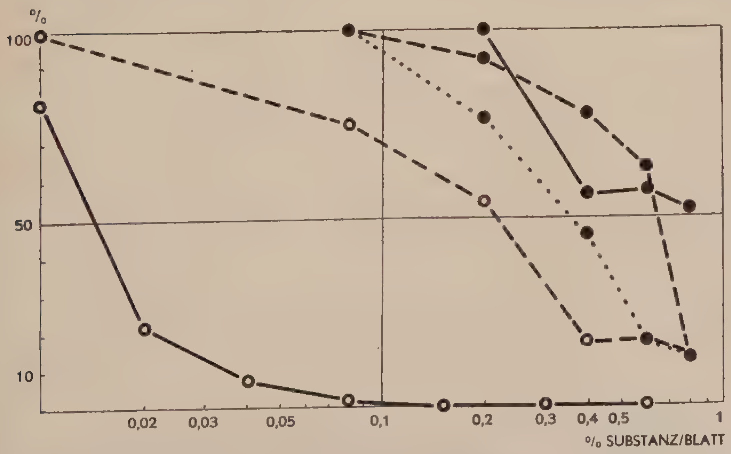


Abb. 9

Abb. 7. Teste mit Tomatin. Gestrichelt: normaler Stamm. Ausgezogen: DDT-resistenter Stamm. Abb. 8. Teste mit Demissin. Gestrichelt: normaler Stamm. Ausgezogen: DDT-resistenter Stamm. Abb. 9. Teste mit verschiedenen Alkaloidglykosiden (normaler Stamm). Leere Kreise und ausgezogene Linie: Präp. 24, amorphes Leptin III. Leere Kreise und gestrichelte Linie: Tomatin. Gefüllte Kreise und gepünktelte Linie: Demissin. Gefüllte Kreise und gestrichelte Linie: α-Chaconin. Gefüllte Kreise und ausgezogene Linie: α-Solanin.

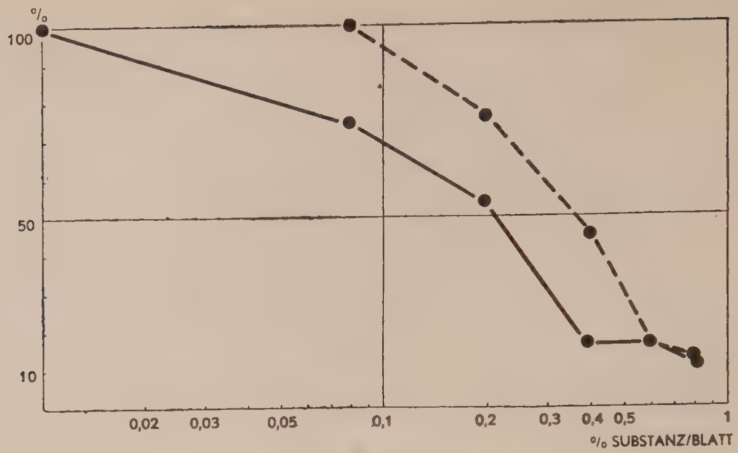
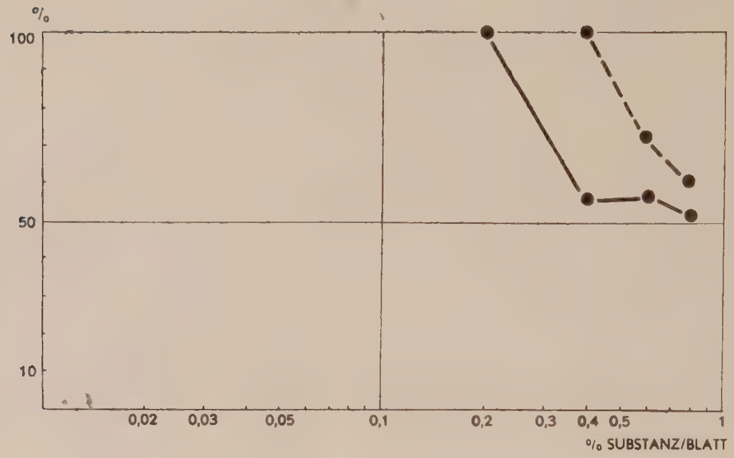


Abb.10



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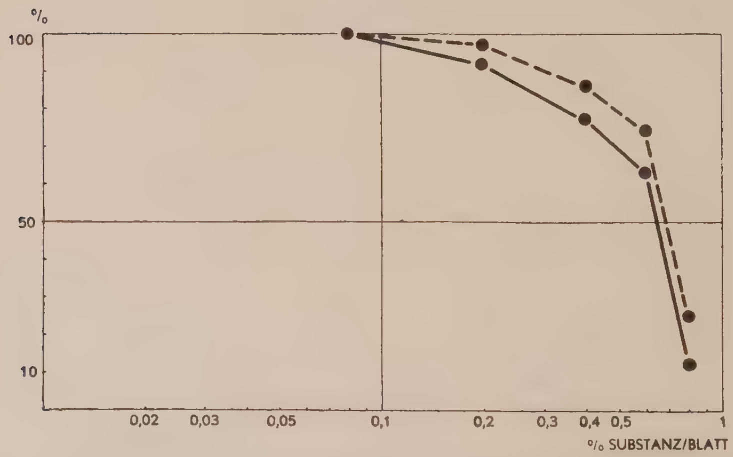

Abb.12

Abb. 10. Teste mit Tomatin und Demissin. Die Kurven (normaler Stamm) von Abb. 7 und 8 sind zusammengestellt. Gestrichelt: Demissin. Ausgezogen: Tomatin. Abb. 11. Teste mit α-Solanin und Leptinin II. Die Kurven (normaler Stamm) von Abb. 1 und 6 sind zusammengestellt. Gestrichelt: Leptinin II. Ausgezogen: Solanin. Abb. 12. Teste mit α-Chaconin und Leptinin I. Die Kurven (normaler Stamm) von Abb. 2 und 5 sind zusammengestellt. Gestrichelt: Leptinin I. Ausgezogen: Chaconin.

DISKUSSION

Die Wirkung der Alkaloidglykoside wird offensichtlich sowohl durch das Aglykon als auch durch den Zuckerrest bestimmt. Abb. 9 zeigt Teste mit Alkaloidglykosiden, die sich von 4 verschiedenen Aglykonen ableiten: dem Solanidin (Solanin und Chaconin), dem Leptinidin (Leptin III), dem Demissidin (Demissin) und dem Tomatidin (Tomatin). Die Abb. 10—12 zeigen Teste mit Alkaloidglykosiden, die jeweils denselben Zuckerrest tragen. Die größte Abhängigkeit der Wirksamkeit von der chemischen Konstitution wurde bei denjenigen Alkaloidglykosiden beobachtet, die sich vom Leptinidin ableiten. Das Vorhandensein der Acetylgruppe ist hier für die Wirksamkeit entscheidend.

Der DDT-resistente Stamm verhielt sich einigen *Solanum*-Alkaloidglykosiden gegenüber andersartig als der normale Stamm. Gegen Alkaloidglykoside, die sich vom Leptinidin ableiten, war er wesentlich unempfindlicher als die normalen Käfer. Auf die Alkaloidglykoside, die sich vom Solanidin ableiten, reagierte er in der gleichen Weise wie die normalen Tiere und gegen Tomatin und Demissin war er empfindlicher als der normale Stamm.

Die statistische Auswertung der Ergebnisse wurde durch die verschiedene Anzahl der Prüfungen je Konzentration erschwert. Wir verzichteten auf sie, weil eine Auswertung gleichartiger Teste (STÜRCKOW, 1959) den Schluß erlaubte, daß die Reaktion des resistenten Stammes auf die Alkaloidglykoside mit dem Aglykon Leptinidin und auf Tomatin und Demissin signifikant ($P < 0{,}1\%$) von dem Verhalten des normalen Stammes verschieden ist.

Herrn Dr. R. Wiesmann von der Fa. Geigy, Basel danken wir sehr für die freundliche Überlassung von 30 DDT-resistenten Käfern.

Knollen vom resistenten *Solanum chacoense*-Stamm, den Fräulein M. Torka jahrelang gehalten hatte, wurden uns vom Max-Planck-Institut für Züchtungsforschung, Rosenhof (Direktor Prof. Dr. E. Knapp) zur Verfügung gestellt, wofür wir herzlich danken.

SUMMARY

THE EFFECT OF SOME SOLANUM *ALKALOIDS ON THE COLORADO BEETLE,* LEPTINOTARSA DECEMLINEATA *SAY*

Different *Solanum* alkaloids (crystallized samples of solanine, chaconine, leptine I, leptinine I, leptinine II, demissine and tomatine, and an amorphous sample of leptine III) were examined by a feeding-test in the Colorado beetle. All were found to discourage uptake of food.

The resistance of *Solanum chacoense* against *Leptinotarsa* is due to its content of leptines. Leptines are transformed into leptinines by loss of the acetyl group. They lose their high activity in this process. Beetles resistant to DDT are less sensitive towards *Solanum chacoense,* leptines and leptinines than normal beetles.

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THE CONTROL OF RELATIVE HUMIDITY WITH AQUEOUS SOLUTIONS OF SODIUM HYDROXIDE

BY

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Using values contained in the International Critical Tables (1928) a gravimetric method, based on one used by SOLOMON (1951), is described to produce a stock solution of sodium hydroxide, from which graded aqueous solutions can be prepared to give relative humidities in steps of 10 per cent between 0° C and 40° C.

Biological laboratory investigations often demand precise control of relative humidities at constant temperatures. These humidities are usually obtained by means of hygroscopic liquids such as aqueous solutions of sulphuric acid, potassium hydroxide (BUXTON & MELLANBY, 1934; SOLOMON, 1951) or glycerol (JOHNSON, 1940), or by saturated salt solutions (reviewed by WINSTON & BATES, 1960). Saturated salts can give only predetermined values of relative humidity, but some aqueous solutions can give relative humidities of any desired value except the very lowest.

Alkalis have the desirable property of absorbing carbon dioxide produced by respiring organisms and so prevent the gas from accumulating in a "closed" apparatus.

SOLOMON (1951) reviewed the various techniques for making up relative humidity solutions and outlined a gravimetric method for preparing graded solutions. He also states that the values used by BUXTON & MELLANBY (1934) to obtain relative humidities with sulphuric acid in aqueous solutions have been superseded by more accurately determined values. Notwithstanding this, the figures published by Buxton & Mellanby are still widely used.

Data in the International Critical Tables make it possible to calculate the weights of sodium hydroxide in aqueous solutions necessary to give any desired humidity and it appeared useful to construct tables from which a solution giving any required relative humidity could be easily obtained.

METHODS AND RESULTS

To obtain any such solution within the temperature range of 0° C—40° C a stock solution of NaOH of known concentration is prepared and weighed aliquots from it are diluted with appropriate weights of distilled water. The various amounts of water to be added to the stock solution were calculated from Solomon's formula. Sodium hydroxide is highly deliquescent so accurate weighing is extremely difficult. Consequently the stock solution was prepared by the technique

TABLE I

The relationship between concentration expressed as weight % and density (to the nearest 0.001) of NaOH solutions at 15° C. (Data from I.C.T. 3, 79).

Weight %	Density	Weight %	Density
1	1.011	22	1.244
2	1.022	24	1.266
3	1.033	26	1.288
4	1.044	28	1.309
5	1.056	30	1.331
6	1.066	32	1.352
7	1.078	34	1.373
8	1.089	36	1.393
9	1.100	38	1.414
10	1.111	40	1.433
12	1.133	42	1.453
14	1.155	44	1.472
16	1.178	46	1.491
18	1.200	48	1.510
20	1.222	50	1.529

described below, which gave satisfactory agreement between results from eight separate batches of stock solutions.

The stock solution was prepared by gradually adding the whole contents of a 500 g jar of NaOH pellets ("Analar") to 550 ml distilled water and shaking until completely dissolved. The specific gravity of the solution was carefully determined at 15° C according to instructions in B.S. booklet 718 (1953) by using a B.S. hydrometer (S.G. 1.500—1.600). Mean results from eight different batches showed that the S.G. was 1.508 (range from 1.505—1.511). These values can be corrected according to (a) B.S. booklet 824 (1938) ± 0.002 for

TABLE II

The relationship between concentration expressed as weight % and vapour pressure (mmHg) of NaOH solutions. (Data from I.C.T. 3, 370).

Concentration of solution		Saturated water vapour pressure (mmHg) and equivalent relative humidities (°/₀)					
Concentration of NaOH (wt. °/₀) /100 g H_2O in solution	Wt. °/₀ of solution (g NaOH/ 100 g solution)	At 0° C		At 20° C		At 40° C	
		e	R.H.°/₀	e_s	R.H.°/₀	e_s	R.H.°/₀
0	0	4.579	100	17.539	100	55.34	100
5	4.76	4.4	96.09	16.9	96.36	53.2	96.1
10	9.09	4.2	91.72	16.0	91.23	50.6	91.4
20	16.67	3.6	78.62	13.9	79.25	44.2	79.8
30	23.08	2.9	63.33	11.3	64.43	36.6	66.1
40	28.57	2.2	48.05	8.7	49.60	28.7	51.8
50	33.33	—	—	6.3	35.92	20.7	37.4
60	37.50	—	—	4.4	25.09	15.5	28.0
70	41.17	—	—	3.0	17.11	10.9	19.7
80	44.44	—	—	2.0	11.40	7.6	13.7
90	48.21	—	—	1.3	7.41	5.2	9.4
100	50.00	—	—	0.9	5.13	3.6	6.5

Concentration (wt. %) of solution and relative humidity (R.H.%) have been calculated from these data to the nearest 0.01.

maximum permissable scale error, and (b) B.S. booklet 824 (1938) ± 0.0012 for surface tension corrections.

The relationship between concentration expressed as weight per cent (from 1—50) and the density of the sodium hydroxide solution at 15° C is given in the International Critical Tables (I.C.T.) (1928). This is shown in Table I.

I.C.T. 3, p. 370 set out the relationship between concentration and vapour pressure of sodium hydroxide solutions. Table II shows this relationship at 0°, 20° and 40° C and includes the corresponding relative humidity values calculated for these temperatures. By combining information in the two tables, relative humidity can be related through V.P. to concentration (wt. %) and concentration can be related to density. Fig. 1 shows this relationship graphically.

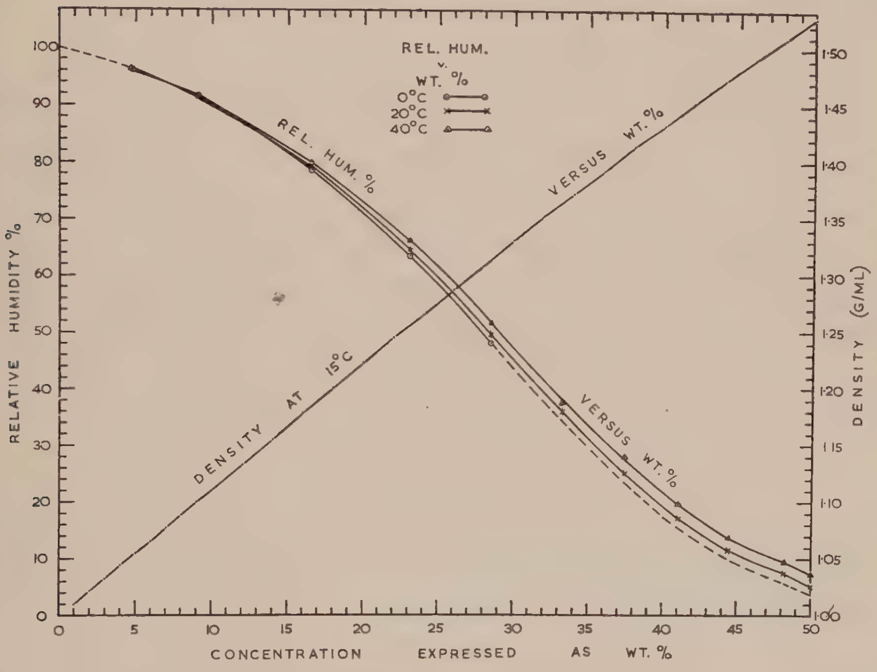

Fig. 1. Graph of data from Table I, showing the relationship between concentration (wt. %) and density of sodium hydroxide solutions at 15° C.
Graph of data from Table II, showing the relationship between concentration (wt. %) and relative humidity over solutions of sodium hydroxide at 0°, 20° and 40° C.

Values relating relative humidity (from 10—95%) and concentration were read off from the graph (see Table III) and data for temperatures other than 0°, 20° and 40° C were derived by linear proportionate interpolation from the calculated relative humidity values. I.C.T. contain too little detail for algebraic equations to be devised for this relationship.

The quantity of water to be added to the stock solution of NaOH to obtain a solution giving a specified relative humidity was calculated from the formula

TABLE III

Concentration (wt. %) required to give specified relative humidity values at 0, 20 and 40° C read off from Fig. I. Data for the remaining temperatures have been calculated from these figures to the nearest 0.01. [N.B. Extrapolation was used for figures below 50% R.H. at 0° C]

R.H.%	Temperature ° C								
	0	5	10	15	20	25	30	35	40
10	44.94	45.14	45.34	45.54	45.74	46.24	46.74	47.24	47.7
20	39.29	39.41	39.57	39.69	39.82	40.07	40.34	40.62	40.8
30	35.03	35.14	35.25	35.36	35.47	35.73	36.02	36.28	36.5
40	31.14	31.26	31.38	31.49	31.61	31.86	32.11	32.36	32.6
50	27.96	28.04	28.12	28.21	28.30	28.50	28.70	28.90	29.1
60	24.43	24.52	24.63	24.71	24.82	25.02	25.21	25.40	25.6
70	20.52	20.62	20.71	20.81	20.89	21.02	21.15	21.28	21.4
80	16.00	16.07	16.14	16.22	16.29	16.36	16.43	16.51	16.5
90	10.06	10.09	10.12	10.15	10.18	10.15	10.13	10.11	10.0
95	5.89	5.89	5.90	5.90	5.91	5.91	5.91	5.90	5.9

$w = \frac{S}{A} - 1$ (SOLOMON, 1951), where w = weight of water in g to be added to each g of stock solution, S = concentration of stock solution and A = the smaller concentration giving the specified relative humidity at a given temperature, both concentrations in wt. %. For simplicity the weights of water (g) to be added to 100 g of stock solution of NaOH (S.G. 1.508 at 15° C; concentration 47.80) were calculated within the range 0° C—40° C to give relative humidities from 10 to 95 per cent (Table IV). Table V shows the densities of the relative humidity solutions from 10—95% at 0° C to 40° C, found by reference to Table III and to the graph of 15° C values (Fig. 1).

TABLE IV

Weight of water (g) to be added to 100 g stock solution of NaOH (S.G. 1.508 at 15° C) to obtain solutions giving specified relative humidities within the range 0° C—40° C

R.H.%	Temperature ° C								
	0	5	10	15	20	25	30	35	40
10	6.4	5.9	5.4	5.0	4.5	3.4	2.3	1.2	0.
20	21.7	21.3	20.8	20.4	20.0	19.3	18.5	17.7	16.
30	36.5	36.0	35.6	35.2	34.8	33.8	32.7	31.8	30.
40	53.5	52.9	52.3	51.8	51.2	50.0	48.9	47.7	46.
50	71.0	70.5	70.0	69.4	68.9	67.7	66.6	65.4	64.
60	95.6	94.9	94.2	93.4	92.7	91.1	89.6	88.2	86.
70	132.8	131.8	130.8	129.7	128.8	127.4	126.0	124.6	123.
80	198.8	197.4	196.2	194.7	193.4	192.2	190.9	189.5	188.
90	375.1	373.2	371.3	369.4	367.5	369.1	370.6	372.2	373.
95	711.5	711.5	710.2	710.2	708.8	708.8	708.8	710.2	710.

The differences in S.G. i.e. 1.505—1.511, which occurred in the eight batches of stock solutions can ultimately lead to variations between the calculated figures and those shown in Table IV. These errors vary with temperature from the

TABLE V

Density values for specified relative humidity solutions from 0° C—40° C read off from graph of 15° C data (Fig. 1)

R.H.%	Temperature ° C								
	0	5	10	15	20	25	30	35	40
10	1.481	1.483	1.485	1.487	1.489	1.493	1.498	1.503	1.507
20	1.426	1.427	1.429	1.430	1.431	1.434	1.437	1.439	1.442
30	1.383	1.385	1.385	1.387	1.388	1.390	1.392	1.395	1.398
40	1.343	1.344	1.345	1.347	1.348	1.350	1.353	1.356	1.359
50	1.309	1.309	1.310	1.311	1.312	1.314	1.316	1.319	1.320
60	1.271	1.272	1.273	1.274	1.275	1.277	1.279	1.281	1.284
70	1.227	1.229	1.230	1.231	1.232	1.233	1.235	1.237	1.238
80	1.177	1.178	1.179	1.180	1.180	1.181	1.182	1.183	1.184
90	1.112	1.112	1.112	1.113	1.113	1.113	1.112	1.112	1.112
95	1.065	1.065	1.065	1.065	1.065	1.065	1.065	1.065	1.065

actual relative humidities, from about ± 1% relative humidity at low values to about ± 0.5% relative humidity at high ones. For accurate results therefore, the specific gravity of the stock solution should be brought as closely as possible to 1.508 at 15° C.

I have much pleasure in thanking Mr. M. E. Solomon for helpful advice and suggestions concerning the manuscript and tables. Miss Maureen Dupuch kindly checked some of the values in the tables. I gratefully acknowledge the receipt of a post-graduate Agricultural Research Council grant which enabled these investigations to be made.

ZUSAMMENFASSUNG

DIE EINSTELLUNG DER RELATIVEN LUFTFEUCHTIGKEIT MIT WÄSSRIGEN LÖSUNGEN VON NATRIUMHYDROXYD.

In der vorliegenden Zusammenstellung werden die Verdünnungsgrade wäßriger Natriumhydroxyd-Lösungen angegeben, die bei Temperaturen zwischen 0° und 40° C bestimmte Stufen relativer Luftfeuchtigkeit in 10%-Intervallen auf Grund der entsprechenden spezifischen Gewichte der Lösungen bedingen. Die Werte sind den internationalen kritischen Tafeln (1928) entnommen und beruhen auf den gravimetrischen Methoden von Solomon (1951).

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ÜBER DIE URSACHEN DER UNTERSCHIEDLICHEN RESISTENZ VON *VICIA FABA* L. GEGENÜBER DER BOHNENBLATTLAUS, *APHIS (DORALIS) FABAE* SCOP.

VII. Reproduktionsrate und Körpergröße von *Aphis fabae* auf gleichaltrigen Jungpflanzen unterschiedlicher Wüchsigkeit¹)

VON

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Reproduktions- und Wachstumsrate von apteren Virginogenien der Schwarzen Bohnenlaus (*Aphis fabae* Scop.) zeigen die bekannten, durch sortenspezifische Antibiosisresistenz der Wirtspflanzen bedingten Unterschiede nicht mehr, wenn Tiere verglichen werden, die an schwachwüchsigen anfälligen (Schlanstedter) *Vicia faba*-Jungpflanzen einerseits und an starkwüchsigen resistenten (Rastatter) anderseits aufgezogen wurden. Dabei erweist sich die Körperlänge der heranwachsenden Jungfern weitgehend proportional zur Größe der (jugendlichen) Blattfläche, die ihnen während ihrer larvalen Entwicklung zur Verfügung stand. Diese hängt ihrerseits sowohl von der Sorte, innerhalb derselben aber auch von der Größe der Samen ab, aus denen die Jungpflanzen aufwuchsen.

Vergleicht man junge, noch wenigblättrige Ackerbohnenpflanzen der blattlausanfälligen Sorte Schlanstedter (S) mit entsprechenden, unter völlig identischen Bedingungen (der Düngung, Temperatur, Luftfeuchtigkeit usw.) aufgezogenen Pflanzen der mehr oder weniger blattlausresistenten Sorte Rastatter (R), so fallen erstere bei genauerer Betrachtung durch etwas kräftigeren Wuchs, besonders in bezug auf Blattgröße und Stengelumfang, auf. Es liegt daher der Verdacht nahe, daß die unterschiedliche Blattlausanfälligkeit (H. J. MÜLLER 1958), soweit sie die antibiotische Resistenz betrifft, wenigstens teilweise mit diesen Unterschieden in der Wüchsigkeit in ursächlicher Beziehung stehen könne. Zu ähnlichen Schlußfolgerungen kommen auch TAMBS-LYCHE & KENNEDY (1958) bei Untersuchungen über die Befallsentwicklung auf verschiedenen Organen (jungen und entfalteten Blättern, Sprossen und Blütentrieben) der zwei Ackerbohnensorten Schlanstedter, Rastatter und einer englischen Tick-Bohne. Sie führen den unterschiedlichen Befall dieser drei Varietäten auf eine sortenweise topographisch verschieden abgestufte Verteilung der Wachstumsintensität in den jugendlichen Geweben zurück.

Unter derartigen Annahmen müßten sich erkennbare Differenzen in der Reproduktionsrate der Aphiden auch schon ergeben, wenn sie auf kräftigeren oder schwächeren Pflanzen ein und derselben Sorte herangezogen werden. Im Herbst 1960 wurde daher in einem behelfsmäßig klimatisierbaren Gewächshaus versucht, solche unterschiedlich wüchsige Jungpflanzen von Rastatter und Schlanstedter

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¹) Quedlinburger Beiträge zur Züchtungsforschung Nr. 47.

Ackerbohnen durch Anzucht aus extrem kleinen und sehr großen Samenkörnern beider Sorten unter sonst gleichen Verhältnissen zu erzeugen. Die dabei deutlich erkennbare Abstufung der Größe (Oberfläche) des ersten Primärblattes und des Stengelquerschnittes sowie ihres prozentualen Zuwachses konnten dann mit der Anzahl und Körpergröße (Länge) von jungen ungeflügelten Virginogenien verglichen werden, die sich, jeweils von einer jungen Mutterlaus produziert, auf den vier Jungpflanzenkategorien (Rastatter und Schlanstedter jeweils aus großen (R bzw. S) und kleinen (r bzw. s) Samen) entwickelten. Dabei sollte sich zeigen, ob eine Beziehung zwischen der Reproduktionskraft der Aphiden und der Wüchsigkeit ihrer Wirtspflanzen bestehe und ob diese gegebenenfalls schon eine hinreichende Erklärung für die unterschiedliche Antibiosisresistenz der beiden Sorten abgeben könne.

MATERIAL UND METHODEN

Die Selektion der jeweils extrem kleinsten und größten Samenkörner aus Rastatter und Schlanstedter Originalsaatgut (Ernte 1959) erfolgte zunächst grob visuell. Dabei wurde jedoch höchstens 1% aus jeweils mehreren Kilo Bohnen ausgewählt, d.h. nur die äußersten Enden der Variationsbreite der Korngrößenstreuung benutzt. Die Tausendkorngewichte des ausgelesenen Versuchssaatgutes betragen:

bei den großkörnigen Schlanstedter Samen	(S)	865,0 g,
bei den kleinkörnigen Schlanstedter Samen	(s)	448,5 g,
bei den großkörnigen Rastatter Samen	(R)	697,5 g,
bei den kleinkörnigen Rastatter Samen	(r)	343,5 g.

Die kleinkörnigen Selektionen beider Sorten wiegen also jeweils nur etwa halb so viel wie die gleichnamigen großkörnigen. Im Vergleich beider Sorten sind aber die Rastatter sowohl der groß- wie der kleinkörnigen Auslese nur um etwa 20 bzw. 23% leichter als die entsprechenden Korngrößen der Schlanstedter. Die Variationsbreiten der Samengewichte von Schlanstedter und Rastatter Ackerbohnen decken sich also nicht, weisen aber ein breites Überschneidungsgebiet auf, so daß die größten Rastatter die kleinsten Schlanstedter noch um mehr als die Hälfte ihres Gewichtes übertreffen und sich nach abnehmenden Gewichten geordnet die Reihe S > R > s > r ergibt, wobei infolge des angewandten Selektionsprinzips die Unterschiede innerhalb der Sorten bedeutend größer sind als zwischen ihnen.

Da die Keimfähigkeit der kleinen Samen erheblich schlechter ist als die der größeren, wurden zunächst viele Samen für 4—6 Tage im Thermostaten bei 27° C zwischen feuchtem Fließpapier vorgekeimt und dann nur diejenigen (einzeln) zur Jungpflanzenanzucht in Blumentöpfen (ø 10 cm) verwendet, die eine normal entwickelte Radicula gebildet hatten. Sobald sich das zweite Primärblatt der jungen Pflanzen entfaltet und das dritte aus dem Sproßgipfel erhoben hatte, wurden die Pflanzen auf das erste Primärblatt zurückgeschnitten und dabei die eine Fiederhälfte desselben sowie die Nebenblätter und die beiden tütenförmigen Vorblätter ebenfalls entfernt, um die Aphiden auf ein möglichst engbegrenztes und dadurch physiologisch vergleichbares, uniformes Substrat zu zwingen. Als wirkliche „Blatt''läuse siedeln sich die Bohnenläuse auf derart gestutzten Pflanzen fast ausnahmslos auf der Unterseite der allein stehengebliebenen Fieder an, solange das Austreiben von Ersatzknospen verhindert wird. Weitere Einzelheiten über die Art der Beschneidung und Aufstellung der Versuchspflanzen in einer Wasserschale zur Sicherung vor Zu- und Abwanderung von Läusen siehe bei H. J. MÜLLER 1958, p. 183, und bei MÜLLER & HENNIG 1960, p. 163. Vielfach, besonders bei R-, r- und s-Pflanzen mußte die Mutterlaus wiederholt angesetzt werden, da sie immer wieder abzuwandern versuchte.

Zur Ermittlung der Blattflächengrößen wurden die Fiederblätter zwischen eine Pappunterlage und transparentes Millimeterpapier geschoben und ihr Umriß mit einem spitzen, weichen Bleistift vorsichtig umfahren. Da das zarte Gewebe leicht beschädigt wird, darf dabei kein Druck ausgeübt werden. Die Größe des Stengelquerschnitts wurde stets in der Mitte zwischen dem zweiten Vorblatt und dem Ansatz des ersten Primärblattes gemessen. Mit Hilfe einer Schublehre mit Nonius, besser mit einer (Zeiss'schen) Mikrometerschraube lassen sich die beiden Querdurchmesser des vierkantigen Stengels hinreichend exakt abgreifen. Ihre Multiplikation ergibt unter der etwas vereinfachenden Annahme eines mehr oder weniger rechteckigen Querschnittes ein praktisch ausreichendes Näherungsmaß für die Querschnittfläche und damit für die Dicke des Stengels. Diese Maße wurden jeweils zu Beginn, d.h. vor dem Ansetzen der jungen Mutterlaus, und am Ende des Versuchs abgenommen, d.h. spätestens, wenn die zuletzt gereifte Tochter ihre letzte Häutung vollendet hatte. Die Differenz beider Werte liefert ein ungefähres Maß für den prozentualen Zuwachs an Blattfläche und Stengeldicke, der während der larvalen Entwicklung der Erstgeborenen-Generationen erfolgte.

Als Ausgangsmütter dienten ausschließlich junge, möglichst erstgeborene ungeflügelte Virginogenien des seit 1948 parthenogenetisch gehaltenen Quedlinburger *Aphis fabae*-Klons, die auf Blättern oder S-Jungpflanzen unter optimalen Ernährungsbedingungen (— ohne Übervölkerung! —) herangezogen worden waren. Diese jungen Mütter wurden einzeln auf die vorbereiteten Jungpflanzen gesetzt und dort bis zur Vollendung der letzten Häutung ihrer zuerst geborenen Tochter belassen (Erstgeborenen-Entwicklungszeit). In diesem Zeitpunkt, d.h. bei 20° C nach durchschnittlich 8 Tagen, wurde die Mutter entfernt und die Anzahl aller inzwischen von ihr produzierten Larven ermittelt (Erstgeborenen-Zahl), während wenigstens die älteren Geschwister der Erstgeborenen bis zu ihrer Imaginalreife dort verblieben. Sobald sich diese nach der letzten Häutung ausgefärbt und ausgehärtet hatten und die wieder beginnende Wachsausscheidung ihre Haut stumpfschwarz erscheinen ließ, erfolgte die Messung ihrer maximalen Länge und Breite unter einem mit Okularmikrometer ausgerüsteten ZEISS-Binokular (PM XVI; Obj. 2,5 ×, Ok. 16 ×). Sie erhielten dazu eine leichte Äthernarkose, so daß sie in normaler Laufhaltung bewegungslos stehenblieben. Ihr Körper weist dann regelmäßig eine weitgehend konstante horizontale Lage auf, so daß die Länge vom frontalen Stirnhöcker bis einschließlich Caudaspitze und die maximale Breite des Abdomens leicht zu messen sind (1 Teilstrich = 0,04 mm). Da sich in einer größeren Stichprobe nachweisen ließ, daß Länge und Breite in enger positiver Korrelation zueinander stehen, wurde bei der Auswertung nur die Körperlänge als Maß für die Größe berücksichtigt.

Insgesamt wurden je Korngrößengruppe etwa 60 Jungpflanzen geprüft, eine entsprechende Anzahl von Erstgeborenen-Generationen aufgezogen und ein Vielfaches von Erstgeborenen gemessen.

ERGEBNISSE

Unterschiede in den Organgrößen der Pflanzen

1. Die Größe der ersten Primärblätter weist zu Beginn des Versuchs, wie die graphische Darstellung (Abb. 1) zeigt, deutlich eine allmähliche Abnahme von S über s und R zu r auf. Im Mittel sind die Blätter der „kleinkörnigen" Jungpflanzen bei beiden Sorten signifikant kleiner als die der „großkörnigen", bei den Schlanstedtern um 14% 1) ($p = 0{,}27\%$), bei den Rastattern um 16,5% ($p < 0{,}10\%$). Viel erheblicher ist jedoch der Unterschied zwischen den Sorten, d.h. die R-Blätter bleiben durchschnittlich um 25% hinter den S-Blättern ($p < 0{,}10\%$) und die r-Blätter um 27% hinter den s-Blättern zurück ($p < 0{,}10\%$). So kommt es, daß die Blätter von s immer noch etwas (10,4%) größer sind als die von R; allerdings ist der Unterschied statistisch nur „genügend" gesichert ($p = 0{,}70\%$). Da die Samengewichte, wie wir oben feststellten, bei R aber erheblich (um 50%) größer waren als bei s, so heißt das, daß wenigstens bis zu Beginn des Versuchs die Blätter der Schlanstedter verhältnismäßig rascher gewachsen sind als die der Rastatter und zwar die s sogar mehr als die S. Infolgedessen ist die bei den Samengewichten bestehende Differenz zwischen R und s bei den Primärblättern zu Versuchsbeginn überkompensiert.

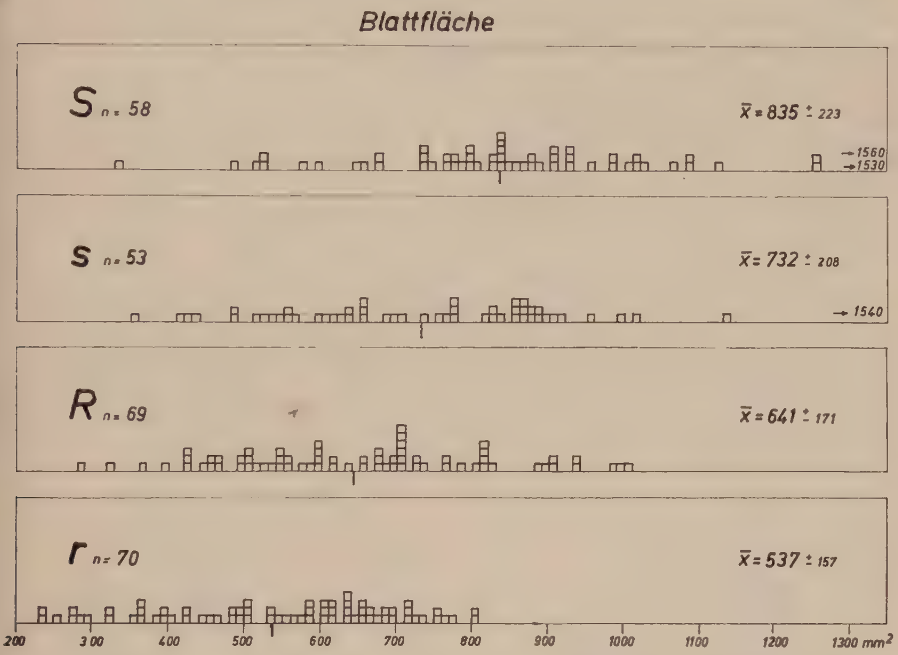

Abb. 1. Größe einer Fieder des ersten Primärblattes (in mm²) von Ackerbohnen-Jungpflanzen, die aus sehr großen (S) und sehr kleinen (s) Schlanstedter und aus sehr großen (R) und sehr kleinen (r) Rastatter Samen herangezogen wurden.

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1) p % gibt die Grenzwahrscheinlichkeit der Nullhypothese über die Differenz zweier Stichproben unter Verwendung des t-Testes nach Student an.

Dieses Bild einer fast kontinuierlichen Abnahme der Blattgröße in der Reihe S > s > R > r verschiebt sich jedoch bis zum Abschluß der Blattlausentwicklung erheblich. Die Unterschiede zwischen großsamigen und kleinsamigen Herkünften der gleichen Sorte haben sich dann stark (etwa um das Doppelte) vergrößert. Im Mittel sind die s-Blätter mit 910,7 ± 233,0 mm^2 nun um mehr als 30% hochsignifikant kleiner als die S-Blätter (1305 ± 308,0 mm^2) und die r-Blätter erreichen mit 667,4 ± 180,0 mm^2 signifikant ebenfalls nur 69,5% der R-Blätter (958,6 ± 224,0 mm^2). Gleichzeitig sind aber auch jetzt noch die Blätter der Rastatter um 27% kleiner als die der Schlanstedter und zwar in beiden Korngrößenherkünften. So hat sich die Spannweite der Differenzen insgesamt vergrößert, zugleich ist aber der Blattgrößenunterschied zwischen R und s völlig ($p = 36{,}8\%$) verschwunden. Im Vergleich mit der Größenfolge S > s > R > r am Beginn des Versuchs zeigt die neue Reihung S > s = R > r am Ende desselben, daß nunmehr das Wachstum der kleinkörnigen Selektionen nachgelassen haben muß.

Am klarsten geht diese unterschiedliche Wachstumstendenz aus einer schematischen Darstellung der absoluten Mittelwerte der Blattgrößen zu Beginn und am Ende der Versuchszeit (d.h. nach 8—10 Tagen) hervor, denen andeutungsweise auch die Tausendkorngewichte der 4 Selektionen zum Vergleich gegenübergestellt sind (Abb. 2). Dabei zeigt sich zunächst die gleichmäßig ansteigende Tendenz in der Größenzunahme der Blätter großsamiger Herkünfte, wobei sich die prozentuale Größendifferenz zwischen R und S allmählich etwas vergrößert. Die kleinen Samen entstammenden Blätter wachsen anfangs stärker (s mehr als r) als die groß-

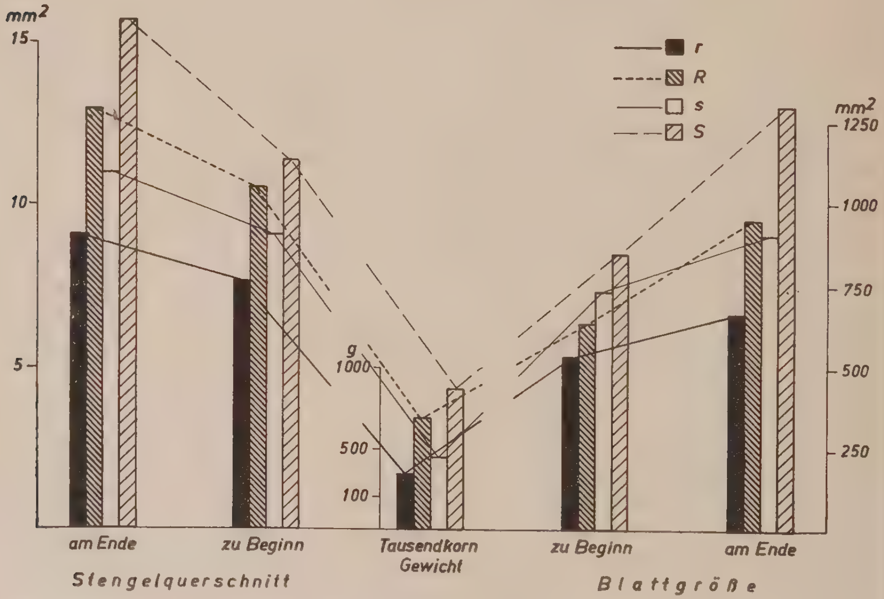

Abb. 2. Schematische Darstellung der absoluten mittleren Blattflächen- (rechts) und Stengelquerschnitts-Größen (links) von Ackerbohnen-Jungpflanzen zu Beginn und am Ende der Erstgeborenen-Entwicklungszeit im Vergleich mit den Tausendkorngewichten der jeweils zur Anzucht benutzten extremen Samengrößenklassen (Mitte).

samigen, so daß es zu der oben erwähnten Verschiebung in der Größenreihe von $S > R > s > r$ der Samen(gewichte) zu $S > s > R > r$ der jungen Primärblätter kommt. Dann aber läßt die Wüchsigkeit der Blätter kleinsamiger Herkünfte stark nach und es tritt in der Reihe eine rückläufige Tendenz zu $S > R \geqq s > r$ ein, die sich wahrscheinlich bei weiterer Entwicklung noch verstärken würde. Allerdings darf nicht vergessen werden, daß sich diese Verhältnisse auf dekapitierte Pflanzen beziehen. Es ist zunächst nicht sicher, ob sie auch bei natürlichem ungestörten Wuchs zutreffen.

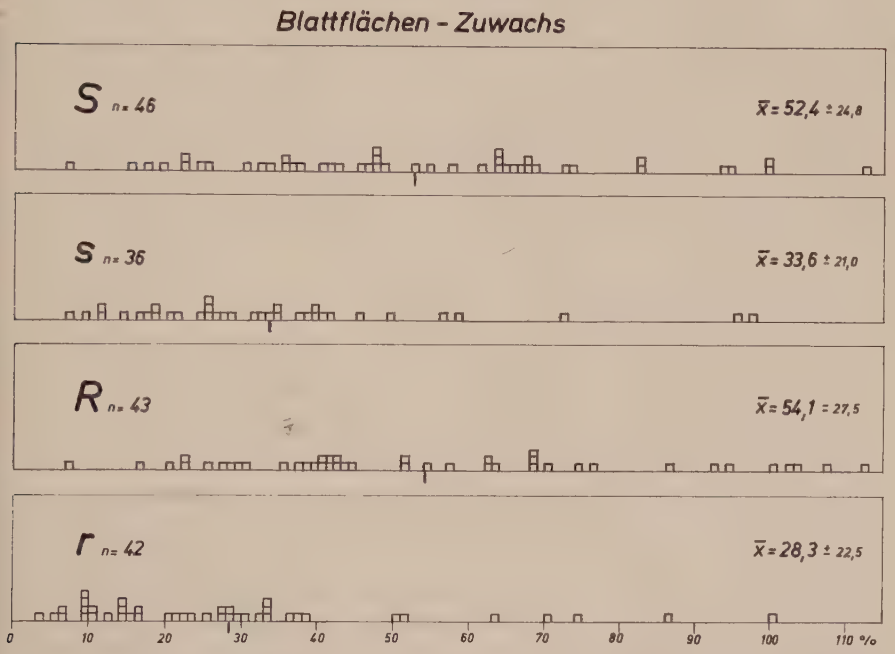

Abb. 3. Zunahme der Blattfläche einer Fieder des ersten Primärblattes von Ackerbohnen-Jungpflanzen während der Erstgeborenen-Entwicklungszeit in Prozent der Anfangsgröße. (Bezeichnungen wie bei Abb. 1).

Vergleicht man den mittleren prozentualen Blattflächenzuwachs (Abb. 3), der bei den einzelnen Jungpflanzenkategorien zwischen Anfang und Ende des Versuchs eintrat, so zeigt sich, daß die Blätter großsamiger Herkünfte praktisch im Mittel gleich stark (S um 52,4 ± 24,8% und R um 54,1 ± 27,5%) zugenommen haben ($p = 76{,}3\%$). Auch die Blätter kleinsamiger Herkünfte zeigen mit $s = 33{,}6 \pm 21{,}0\%$ und $r = 28{,}3 \pm 22{,}5\%$ keinen signifikanten Sortenunterschied ($p = 27{,}1\%$) ihrer Zuwachswerte, aber ihre Vergrößerung blieb sehr erheblich (bei s um 36,0% und bei r um 47,8%) hinter dem Flächenzuwachs der großsamigen Pflanzen zurück. Der prozentuale Blattzuwachs ergibt also die Reihe $S = R > s = r$.

2. Zwar leben und entwickeln sich die *Aphis fabae*-Kolonien unter den Versuchsbedingungen fast ausnahmslos auf den Blättern, doch sollte geprüft werden, ob nicht auch Umfang und Wachstum des Stengelteiles, der die befallenen Blätter

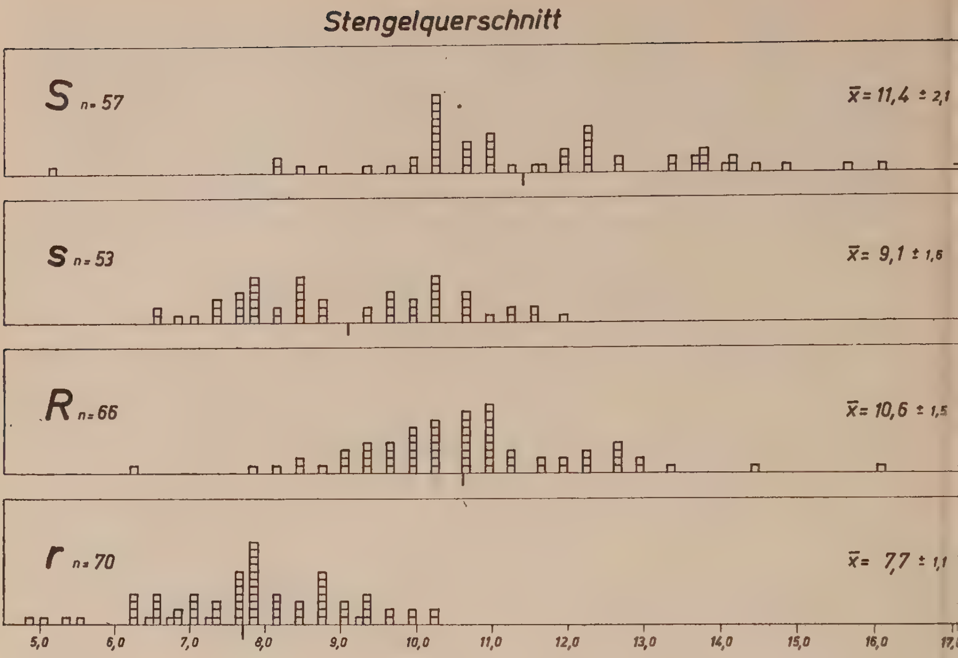

Abb. 4. Querschnittsgröße der Stengel (in mm^2) junger Ackerbohnenpflanzen, die aus Samen verschiedener Größe angezogen wurden. (Bezeichnungen wie bei Abb. 1).

trägt, eine Bedeutung für die Blattlausreproduktion haben könnte. Zu Versuchsbeginn weisen die aus kleinkörnigen Samen erwachsenen Stengel beider Sorten um 20—28% kleinere Querschnitte auf als die aus großen Samen erzogenen (p in beiden Fällen $< 0{,}10\%$), wie die Abb. 4 zeigt. Der Sortenabstand bei Pflanzen aus Samen der entsprechenden Größenkategorie ist dagegen deutlich geringer: zwischen S und R mit nur 7,0% nur eben „genügend" signifikant ($p = 0{,}52\%$) und zwischen s und r 15,5% ($p < 0{,}10\%$). Die Größenfolge der Stengeldicken entspricht mit $S > R > s > r$ demnach völlig jener der Samengewichte, d.h. also der Größe der Samen, aus denen sie hervorgingen, wobei die „kleinsamigen" Pflanzen jedoch etwas „aufgeholt" haben, so daß sich die Unterschiede prozentual eher etwas vermindert haben (siehe auch Abb. 2).

Im weiteren Verlauf der Entwicklung bleiben wie bei den Blättern die Stengel aus kleinen Samenkörnern im Wachstum stark hinter denjenigen aus großen zurück. Ihre Querschnitte sind nun sowohl bei s wie bei r um 30,0% kleiner ($p < 0{,}10\%$) als bei den großkörnigen Selektionen der gleichnamigen Sorten. Zugleich hat sich aber auch der Abstand zwischen den Sorten vergrößert, besonders zwischen S (mit $\overline{x} = 15{,}7 \pm 2{,}4$ mm^2) und R (mit $\overline{x} = 13{,}0 \pm 1{,}6$ mm^2), wo er nun infolge verringerten Wachstums der R-Pflanzen 17,0% beträgt ($p < 0{,}10\%$), weniger zwischen s ($\overline{x} = 11{,}0 \pm 1{,}8$ mm^2) und r ($\overline{x} = 9{,}1 \pm 1{,}6$ mm^2), wo er von 15,5 nur auf 17,3% anstieg. Obwohl sich also die Abstände zwischen den durchschnittlichen Stengeldicken der vier Pflanzengruppen — wie bei den Blättern — während der Versuchszeit erheblich vergrößert haben, ist doch die Reihenfolge der Stengelquerschnittgrößen $S > R > s > r$ unverändert erhalten geblieben.

Der prozentuale Dickenzuwachs der dreidimensional und dabei zweifellos bevorzugt in der — hier nicht berücksichtigten — Längsrichtung wachsenden Stengel ist naturgemäß viel geringer als die Flächenzunahme der Primärblätter (Abb. 5). Die geringe Zuwachsrate der R bedingt für den Stengelzuwachs die Reihenfolge S > s = R > r, da den geringen Differenzen zwischen s und R keine Signifikanz zukommt.

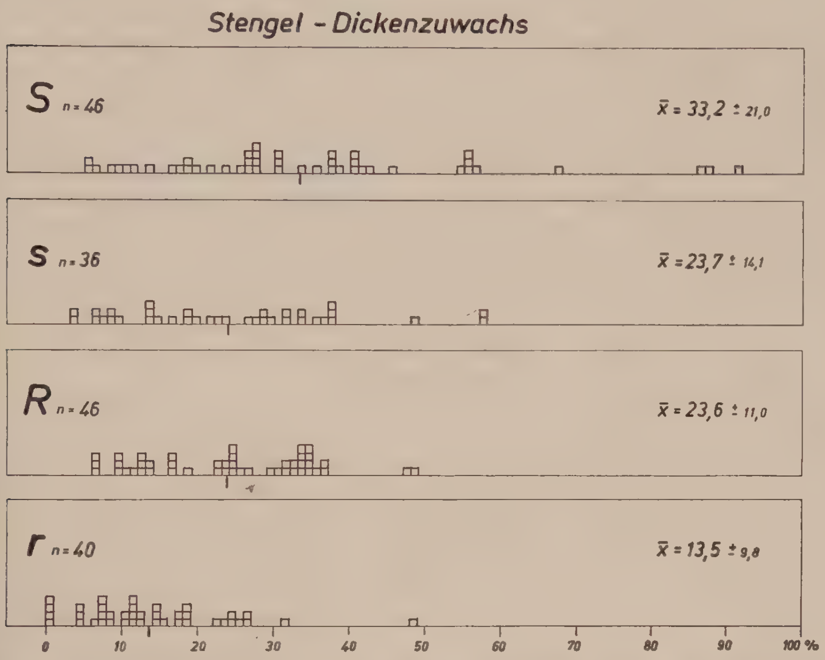

Abb. 5. Zunahme des Stengelquerschnitts junger Ackerbohnen während der Erstgeborenen-Entwicklung in Prozent der Anfangsgröße.

Jeweils sehr kleine und sehr große Samenkörner (Gewichtsdifferenz 50%) der beiden Ackerbohnensorten Schlanstedter und Rastatter liefern also Jungpflanzen entsprechend unterschiedlicher Größe und Wüchsigkeit. Bei beginnender Entfaltung des dritten Primärblattes sind die Flächen des ersten Primärblattes und des Stengelquerschnitts unterhalb desselben bei Pflanzen aus großen Samen stets erheblich (15—25%) größer als bei solchen aus kleinen. Diese Unterschiede vergrößern sich innerhalb der nächsten 8—10 Tage trotz Dekapitation der Pflanzen (über dem ersten Primärblatt) noch weiter auf ca 30%. Dies beruht offensichtlich auf einer allgemein verminderten Wüchsigkeit der Pflanzen, die aus kleinen Samen erwachsen, deren Blattfläche sich zwar zunächst relativ rascher vergrößert, dann aber (in der Versuchszeit) nur noch viel weniger zunimmt als diejenige der „großsamigen".

Der daneben schon bei dem Gewicht der Samenkörner bestehende Unterschied von 20—25% zwischen den beiden Varietäten bleibt bei den Blattflächen annähernd erhalten, während er sich bei den Stengeldicken eher vermindert. Lediglich ein anfänglich überdurchschnittliches Wachstum der Blätter von s-Pflanzen, das vorübergehend sogar zu einem Überflügeln der R-Blattgrößen führt, und ein frühzeitiges Nachlassen des Stengelwachstums bei R-Pflanzen, das eine Angleichung der Stengelzuwachsrate zwischen R und s bedingt, scheinen hinsichtlich der Wüchsigkeit sortenspezifisch zu sein.

Unterschiede in der Anzahl und Größe der Bohnenläuse

1. Der Nachweis eines antibiotischen Effektes von Rastatter Ackerbohnen auf die Reproduktionsrate von *Aphis fabae* ist bereits früher und wiederholt (H. J. Müller 1958, Müller & Hennig 1960) geführt worden. Die Häufigkeitsverteilung der auf S, s, R und r von jeweils etwa 40 apteren Jungfern erzeugten Erstgeborenen-Zahlen bestätigt zunächst diese Erfahrung (Abb. 6). Ganz gleichgültig, ob als Nährpflanzen Keimlinge aus sehr großen oder sehr kleinen Samenkörnern dienten, die Anzahl der — zwischen Geburt und Imaginalreife der zuerst geborenen Tochter — erzeugten Nachkommen liegt im Mittel auf Schlanstedter um 7 bis 8,5 Individuen, d.h. rund 20% höher als auf Rastatter. Trotz der erheblichen Streuung sind diese Unterschiede statistisch signifikant ($p < 0,10\%$).

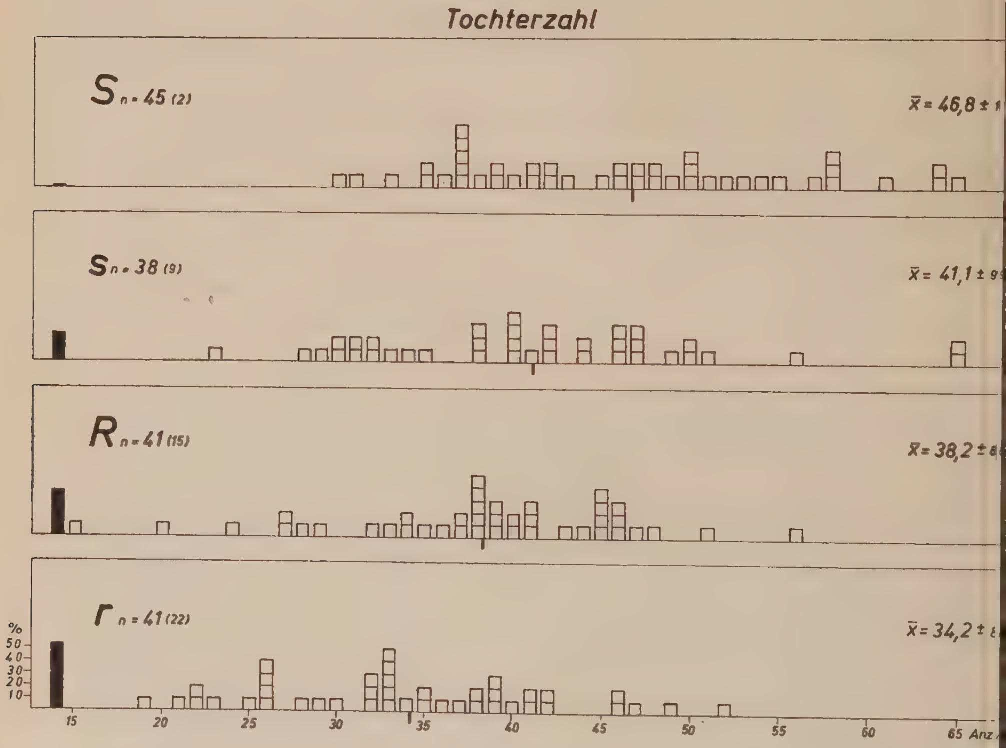

Abb. 6. Häufigkeitsverteilung der Nachkommenzahlen, die aptere virginogene *Aphis fabae* zwischen Geburt und Reife ihrer erstgeborenen Töchter auf Primärblättern von Ackerbohnen-Jungpflanzen (aus verschieden großen Samen) produzierten. Schwarze Säulen (links) = Prozentsatz der in der Darstellung enthaltenen „unvollständigen" Tochterfamilien. (Abkürzungen wie bei Abb. 1).

In dieser Aufstellung sind auch die sog. unvollständigen Tochterfamilien enthalten, deren Mütter mehr oder weniger lange vor der Reife ihrer erstgeborenen Tochter die Produktion weiterer Larven einstellten oder starben, obwohl dies auf Rastatter stets viel mehr sind als auf Schlanstedter und damit eine ungleiche Belastung der Rechnung entsteht. Jedoch scheint gerade dies der beste Weg, um eben die charakteristische Vitalitäts- und damit Reproduktionsminderung der auf Rastatter lebenden Läuse mit in Ansatz zu bringen. Diese frühzeitige Sterilität bzw. Mortalität der jungen Mütter nimmt in den vorliegenden Zuchtserien in der Reihe S — s — R — r von 4,4 über 23,5 und 36,5 bis zu 53,5% aller angesetzten Jungfern erheblich zu (siehe Abb. 6). Dabei sind die Unterschiede innerhalb der Sorten (zwischen stark- und schwachwüchsigen Pflanzen) viel geringer als zwischen den Sorten (bei Pflanzen aus gleichen Samenkategorien). Demnach ergibt sich auch hier eine deutliche Annäherung der Werte von s und R.

Die gegenüber der ersten Veröffentlichung (H. J. Müller 1958) gering erscheinende Differenz in den Nachkommenzahlen auf Schlanstedter und Rastatter (damals = 11,2, jetzt 8,6 bzw. 6,9) entsteht offensichtlich dadurch, daß damals die Ergebnisse von je 25 genetisch zusammenhängenden, aufeinanderfolgenden Generationen (in je fünffacher Wiederholung) zusammengefaßt worden waren, während hier wie in umfangreichen ähnlichen Experimenten (H. J. Müller, unveröffentlicht) jeweils für jeden Einzelversuch frische Jungweibchen aus optimal ernährten Stammzuchten benutzt und aus vielen Einzelzuchten die Mittelwerte berechnet wurden. Der antibiotische Effekt verstärkte sich damals von Generation zu Generation, weil die Nachkommen stets auf die gleiche Sorte, immer wieder auf R oder immer wieder auf S gesetzt wurden. Hier dagegen bleibt er relativ schwach; denn in jeder neuen Versuchsgeneration wurden die Erstgeborenen als Embryonen größtenteils bereits zu einer Zeit gebildet, in der ihre Mutter noch optimale Ernährungsbedingungen hatte.

Den relativ großen Unterschieden in der Erstgeborenen-Zahl auf den Sorten stehen ähnliche, wenn auch nicht ganz so starke zwischen den aus großen und kleinen Samen einer Varietät herangezogenen Pflanzen gegenüber: im Mittel wurden auf s 5,7 (= 12,3%) weniger Larven produziert als auf S ($p = 0{,}92\%$) und auf r 4,0 (= 10,5%) weniger als auf R ($p < 0{,}10\%$). Der geringe Unterschied zwischen den Erstgeborenen-Zahlen von s (41,1 ± 9,6) und R (38,2 ± 8,3) ist statistisch aber nicht mehr signifikant ($p = 16{,}0\%$), so daß sich hinsichtlich der Tochterzahl die Reihe $S > s = R > r$ ergibt.

Die antibiotische Wirkung auf die Reproduktionsrate der Bohnenläuse verschwindet demnach, wenn Jungpflanzen aus extrem kleinkörnigen Samen der anfälligen mit solchen aus extrem großen Samen der resistenten Varietäten verglichen werden, während gleichzeitig deutliche Unterschiede bei Jungpflanzen aus verschieden großen Samen innerhalb der gleichen Sorte in Erscheinung treten. Mindestens ein Teil des antibiotischen Effektes dürfte demnach einfach die Folge unterschiedlicher Größe bzw. Wüchsigkeit der Wirtspflanzen sein (s.u.).

2. Wenn das der Fall ist, so wäre zu vermuten, daß sich auf verschieden kräftigen Pflanzen auch schon Unterschiede in der Wüchsigkeit der Blattläuse selbst zeigen müßten, zumal wenn man bedenkt, daß auch die Reproduktionsrate letztlich auf einem Wachstumprozeß beruht. Als Maß für das postembryonale Wachstum

wurde hier die Größe (Körperlänge) benutzt, welche die Erstgeborenen nach der Häutung zur Imago erreichten (Maßeinheit = 0,04 mm).

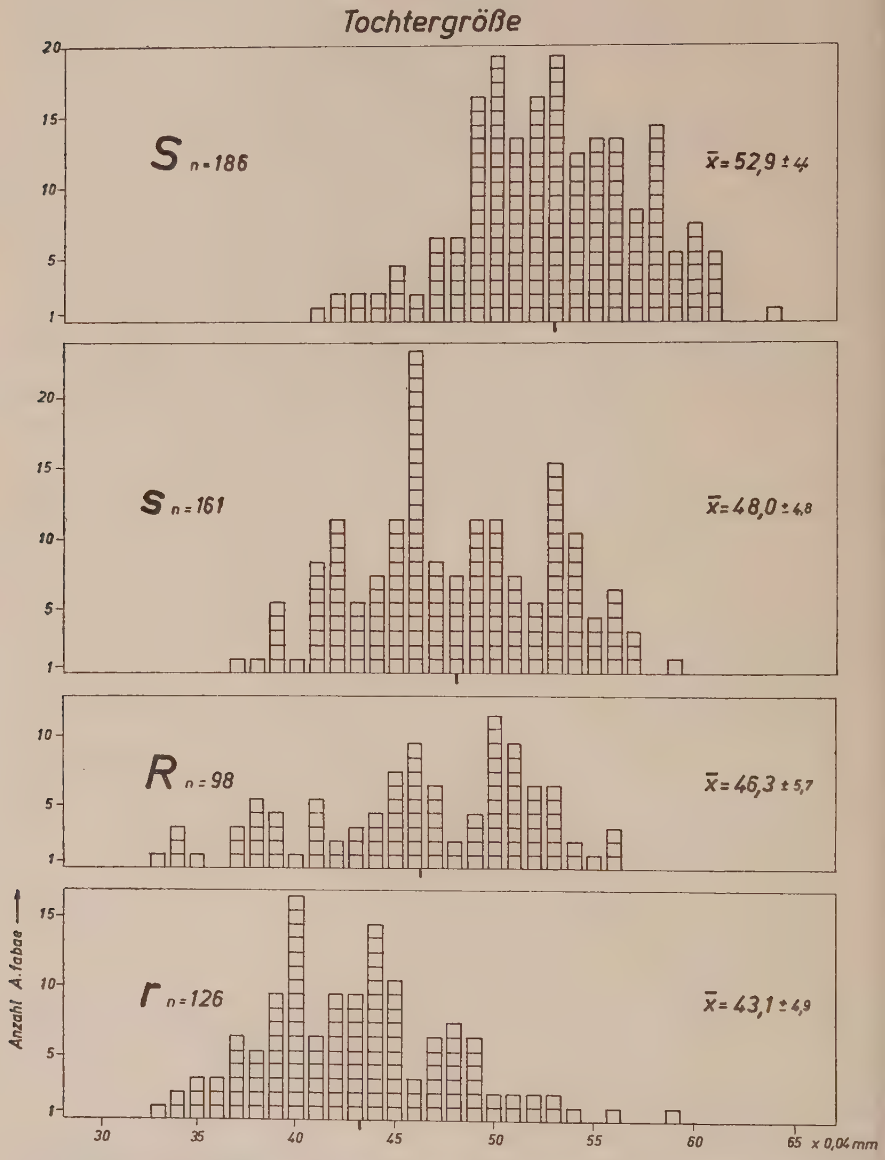

Abb. 7. Häufigkeitsverteilung der Körperlängen (Stirnhöcker - Caudaspitze) erstgeborener Töchter apterer virginogener *Aphis fabae*, die sich auf Primärblättern von Ackerbohnen-Jungpflanzen (aus Samen verschiedener Größe und Sorte) entwickelten.

Dabei ergeben sich tatsächlich ganz ähnliche Verhältnisse wie bei der Reproduktionsrate (Abb. 7). Auch hier ist der Unterschied zwischen den Läusen, die auf Jungpflanzen aus verschieden großen Samen einer Sorte heranwachsen, groß und statistisch signifikant ($p < 0{,}10\%$). Die Erstgeborenen werden auf S im Mittel um 12% länger als ihre Geschwister auf s, und die auf R herangewachsenen übertreffen die auf r entstandenen um nahezu 8%. Dagegen besteht zwischen den Läusen von s- und R-Pflanzen nur noch eine Längendifferenz von 2% ($p = 0{,}70\%$). Auch die Wachstumsrate der Larven ergibt also eine ähnliche Größenfolge ($S > s \geqq R > r$) wie die Reproduktionszahl, wobei allerdings die Differenz zwischen den Läusen, die auf „kleinkörnigen'' Pflanzen der anfälligen Sorte herangewachsen sind, und jenen von „großkörnigen" Pflanzen der resistenten Sorte ebenfalls nur sehr gering, statistisch aber genügend wahrscheinlich ist. Im Mittel ergeben zwar alle, selbst die aus kleinen Körnern erwachsenen Schlanstedter Pflanzen noch größere Jungläuse als die Rastatter. Dennoch bestärkt die geringe Differenz zwischen R- und s-Läusen und die größeren Abstände von S zu s und R zur r die Vermutung, daß der antibiotische Effekt weitgehend von der Größe bzw. Wüchsigkeit der Wirtspflanzen abhängig sei.

Zugleich erweist sich die im Verlauf der postembryonalen Entwicklung erreichte Größe der Bohnenläuse als ein günstigeres Maß für den antibiotischen Effekt als die Nachkommenzahl. Während für die Größenbestimmung schon die Anzahl von Tieren genügt, die in einer Generation unter identischen Bedingungen (eventuell auf nur einer Pflanze) heranwachsen, müssen zur Entwicklung eines statistisch ausreichenden Materials viele Generationen aufgezogen werden, die oft schon aus räumlichen Gründen nicht gleichzeitig und in jedem Falle nur auf verschiedenen Pflanzen ablaufen können. Infolgedessen ist die Streuung der Einzelwerte hierbei meist doppelt so groß wie bei den Körperlängen.

TABELLE I

Größenordnung von Rastatter und Schlanstedter Ackerbohnen-Jungpflanzen, die aus großen und kleinen Samen aufwuchsen, in bezug auf

1.	das Tausendkorngewicht ihrer Samen	$S > R > s > r$
2.	ihre Blattgröße	
	a) zu Beginn des Versuchs	$S > s \underset{(=)}{>} R > r$
	b) am Ende des Versuchs	$S > R = s > r$
3.	den prozentualen Zuwachs ihrer Blattfläche	$S = R > s = r$
4.	ihre Stengelquerschnittgröße	
	a) zu Beginn des Versuchs	$S > R > s > r$
	b) am Ende des Versuchs	$S > R > s > r$
5.	ihren prozentualen Stengelquerschnitt-Zuwachs	$S > R = s > r$
6.	a) die Anzahl (Erstgeborenen-Zahl)	$S > s = R > r$
	b) die Größe (Erstgeborenen-Größe) der sich auf ihnen entwickelnden apteren Bohnenläuse	$S > s \underset{(=)}{>} R > r$
7.	die Sterblichkeit der Blattlausmütter auf ihren Blättern	$S < s < R < r$

Pflanzenwachstum und Blattlausvermehrung

Die möglichen Beziehungen zwischen Größe und Wachstum der Jungpflanzenorgane auf der einen, Anzahl und Größe der auf ihnen erwachsenen Virginogenien auf der anderen Seite sind schon weitgehend aus dem Vergleich der graphischen Darstellungen (Abb. 1 bis 7) sowie aus den Differenzreihen der vorangehenden Abschnitte abzulesen (Tabelle I). Dabei ergeben sich im wesentlichen folgende parallele Beziehungen. Der Größenanordnung der verwendeten Korngewichtsklassen ($S > R > s > r$) entsprechen mit der gleichen Reihenfolge die Durchmesser der aus ihnen hervorgehenden Jungpflanzensprosse, sowohl am Anfang wie am Ende der Versuchsperiode. Die Berechnung des prozentualen Dickenzuwachses ergibt aber schon eine Angleichung der mittleren Glieder, d.h. die Folge $S > R = s > r$. Diese ist sowohl für die Folge der Blattgrößen am Ende des Versuchs wie für die Reihung der Erstgeborenen-Zahlen, die sich auf ihnen entwickelten, charakteristisch. Bei den Anfangsblattgrößen und den Körpergrößen der von ihnen ernährten Erstgeborenen setzt sich diese Tendenz in einem Austausch der mittleren Glieder fort, so daß nunmehr die Reihenfolge $S > s \geq R > r$ entsteht, bei der freilich die Differenz $s > R$ statistisch nicht so signifikant ist wie die zwischen den anderen Gliedern. In gleicher Reihenfolge nimmt umgekehrt die Sterblichkeit bzw. Sterilität der Mutterläuse zu.

Während der Entwicklung der jungen Ackerbohnenpflanzen verschieben sich also offensichtlich infolge unterschiedlicher Wachstumsgeschwindigkeit der beiden Sorten die Größenverhältnisse ihrer Organe. Für die absoluten Werte der Stengeldurchmesser des untersten Internodiums wirkt sich die Beschleunigung des Wachstums der Schlanstedter innerhalb des Versuchszeitraumes noch nicht aus, d.h. sie zeigen noch die gleiche Stufenfolge wie die selektierten ursprünglichen Samengrößen (Tausendkorngewichte), doch im prozentualen Dickenwachstum schieben sich die aus kleinen Samen erwachsenen Schlanstedter schon auf das Niveau der Rastatter aus großen Samen. Den gleichen Zustand zeigen die Blattgrößen am Ende des Versuchs, während vorher die „kleinsamigen" s die „großsamigen" R an Fläche sogar übertroffen hatten. Die anfängliche Beschleunigung des Wachstums der Schlanstedter Ackerbohnen scheint also später wieder etwas nachzulassen, so daß der prozentuale Blattzuwachs in der Versuchsperiode für die „großsamigen" und „kleinsamigen" Pflanzen ausgeglichen ist ($S = R > s = r$); doch vergrößert sich die Spannweite der absoluten Differenzen bis zuletzt, so daß sich die Variationsgrenzen in der Organgröße beider Sorten mindestens vorübergehend stärker auseinanderschieben (s. Abb. 2).

Vitalität und Reproduktionspotenz der Mutterläuse, die auf diesen wüchsigen Jungpflanzen leben, sowie die Wachstumsrate ihrer erstgeborenen Töchter, die sich auf ihnen entwickeln, spiegeln deutlich die unterschiedliche Wachstumstendenz der Wirtspflanzen wieder. Dabei entspricht die Reproduktionsrate (Erstgeborenen-Zahl) dem von Stengelzuwachs und Blattgröße zuletzt erreichten Zustand, u.a. derart, daß Unterschiede zwischen R und s nicht mehr signifikant, antibiotische Resistenzeffekte mithin in diesem Bereich nicht mehr nachweisbar sind. Die Vitalität der Mutterläuse wie die Wachstumsrate ihrer Töchter folgen dagegen den maximalen Wachstumsunterschieden, welche die jungen Primärblätter am Beginn der Versuche aufwiesen. Die Ursachen der unterschiedlichen antibiotischen Resistenz der Rastatter und Schlanstedter Ackerbohnen scheinen daher offensichtlich in erster Linie in der unterschiedlichen Wachstumsintensität dieser beiden Sorten zu liegen.

Da sich Wirtspflanzen wie Aphiden während des Versuchs lebhaft entwickelten, d.h. sowohl die als Substrat dienenden Pflanzenorgane wie die Anzahl und Größe

TABELLE II

Beziehungen zwischen Blattfläche und Blattlauszahl und -größe.

	a	b	c	d	e
Sorten und Samengrößen	mittlere Anfangs-Blattgröße in mm^2	mittlere Erstgeborenen-Tochterzahl	Blattfläche pro Larve in mm^2 $(\frac{a}{b})$	mittlere Blattlausgröße	Quotient $\frac{d}{c}$, d.h. $\frac{\text{Blattlausgröße}}{\text{Blattfläche/Larve}}$
S	853	46,8	18,1	52,9	2,92
s	732	41,1	17,8	48,0	2,70
R	641	38,2	16,8	46,3	2,74
r	537	34,2	15,7	43,1	2,74

der Jungläuse ständig wuchsen, läßt sich eine offensichtlich zwischen den beiden Wachstumsprozessen beider Partner bestehende Korrelation nur schwer erfassen. Wenn man aber davon ausgeht, daß beide Vorgänge die gleiche positive Tendenz haben und sich parallel entwickeln, scheint es berechtigt, die ermittelten Werte, z.B. Blattgröße und Aphidengröße, quasi als momentan stationäre Augenblicksbilder dieser Prozesse bzw. als Ergebnisse der bis dahin abgelaufenen Entwicklung zu betrachten und zu vergleichen. So lag es nahe zu prüfen, wieviel Blattfläche auf die einzelne, sich entwickelnde Junglaus entfiel. In Tabelle II sind in Spalte c die Werte ersichtlich, die sich aus einer entsprechenden Division der mittleren (Anfangs-)Größe der Blattfläche durch die mittlere Erstgeborenen-Anzahl für die vier Jungpflanzengruppen ergeben. Es entsteht wieder die Reihenfolge $S > s > R > r$, d.h. bei S entfällt auf jede sich entwickelnde Larve mehr Blattfläche als bei s, bei dieser mehr als bei R usw. Danach lassen sich die Größenunterschiede der zur Imago herangewachsenen Töchter als Folge der unterschiedlichen Substratmenge betrachten, die ihnen während ihrer postembryonalen Entwicklung zur Verfügung stand. Wenngleich sich aus diesem Befund auch die Notwendigkeit noch weiterer Nachprüfung ergibt, so scheint wenigstens eine überschlagsweise Berechnung des Quotienten $\frac{\text{Blattlausgröße}}{\text{Blattfläche/Larve}}$ schon am vorliegenden Material möglich. Trotz der einschränkenden Unvollkommenheit der verfügbaren Unterlagen (Mittelwerte mit großen Streuungen) ergibt sich in allen vier Fällen ein ganz ähnlicher Wert (Spalte e), d.h. das Verhältnis zwischen Blattlausgröße und pro Larve verfügbarer Blattfläche ist für alle vier Pflanzengruppen — sowohl für die Sorten wie für ihre Korngrößenherkünfte — weitgehend konstant. Oder anders ausgedrückt: die Imaginalgröße einer Bohnenlaus ist etwa proportional der Blattfläche (jungen Gewebes), die ihr bei der Entwicklung als Substrat diente, wobei sich ein Proportionalitätsfaktor von $c = 2{,}8$ berechnet.

DISKUSSION

Die durch antibiotische Resistenzmechanismen hervorgerufenen Unterschiede in der Reproduktions- und Wachstumsrate zwischen Schwarzen Bohnenläusen von anfälligen (Schlanstedter) und resistenten (Rastatter) Ackerbohnen sind nicht mehr nachweisbar, wenn Populationen von schwachwüchsigen, relativ kleinblättrigen Schlanstedter Jungpflanzen mit solchen von starkwüchsigen und relativ großblättrigen Rastattern verglichen werden. Da außerdem zumindesten die Wachstumsrate der Erstgeborenen auf jungen wüchsigen Blättern der Blattgröße etwa proportional zu sein scheint, liegt es nahe anzunehmen, daß die Antibiosiseffekte der Ackerbohnenresistenz weitgehend auf Unterschieden in der Wüchsigkeit der Sorten, vermutlich also eher auf quantitativen als auf qualitativ sortenspezifischen Eigenschaften beruhen. Nur insofern die Sorten in diesem Stadium ihrer Entwicklung durchschnittlich größere oder kleinere Organe produzieren — was freilich eine Folge ihrer Wüchsigkeit, ihres endogenen ontogenetischen Rhythmus sein mag —, liegt die Ursache der Resistenz in ihnen; aber nur in dem Sinne, daß die Variationsbreite des gleichen wirksamen Prinzips bei beiden in verschiedener Richtung verschoben ist. Es lassen sich jedoch durch einfache Größenselektion in beiden Sorten Pflanzenindividuen finden, die untereinander identische Bedingungen für die Entwicklung der Aphiden bieten und also keine Resistenzunterschiede mehr zeigen. Es bleibt zu prüfen, ob sich etwa auf flächengleichen Blättern beider Sorten — die sich aus Jungpflanzen aus großen Samen von Rastatter und kleinen von Schlanstedter herauslesen lassen — im Mittel stets gleich große und gleich viele Erstgeborene entwickeln. Nur auch dann noch bestehende Unterschiede würden echte sortenspezifische Resistenz darstellen.

Da in den vorliegenden Untersuchungen den Blattlausmüttern wie den heranwachsenden Larven alters- und entwicklungsmäßig identische und stoffwechselphysiologisch gleich behandelte Blattsubstrate zur Verfügung standen, können die beobachteten Unterschiede in ihrer Reproduktions- bzw. Wachstumsrate nicht auf Differenzen zurückgeführt werden, wie sie Kennedy und Mitarbeiter in den letzten Jahren an Blättern unterschiedlichen Alterszustandes, verschiedener Hydratatur und assimilatorischer Aktivität nachgewiesen haben. Sie entsprechen vielmehr den Ergebnissen von Auclair & Cartier (1960) an Erbsenläusen, deren Gewichte an identischen Organen resistenter und anfälliger Erbsensorten entsprechende Unterschiede aufwiesen. Ähnliche Unterschiede in der Reproduktions- und Wachstumsintensität konnten auch erzeugt werden, wenn auf anfälligen Sorten gehaltene Erbsenläuse täglich 10—12 Stunden hungern mußten. Dies spricht in Übereinstimmung mit dem hier erbrachten Nachweis einer Abhängigkeit der Wüchsigkeit der Bohnenläuse von der Größe der zur Verfügung stehenden Substratfläche dafür, daß die antibiotische Resistenz bei Blattläusen weitgehend quantitative Ursachen haben muß. Daß auch qualitative biochemische Unterschiede eine Rolle spielen, haben Maltais & Auclair (1957) ebenfalls an *Acyrthosiphon pisum* (Harr.) wahrscheinlich gemacht. Es wird in Zukunft notwendig sein zu prüfen, inwieweit die quantitativen Effekte einerseits nur eine Funktion der für die einzelne, sich entwickelnde Blattlaus zur Verfügung stehenden Substratfläche darstellen, und in welchem Maße sie andererseits wirklich dem prozentualen Anteil bestimmter Nahrungsstoffe in ihr entsprechen.

Seit den Arbeiten Mittlers (1957, 1958) an *Tuberolachnus salignus* Gmelin hat sich die Vorstellung entwickelt, daß die Nahrungsaufnahme der Aphiden weitgehend passiv und mehr oder weniger eine Funktion des in den Siebröhren herrschenden Druckes sei. Im Falle der Bohnenläuse, die sich auf dem einzigen, einer dekapitierten Pflanze belassenen Primärblatt entwickeln, erhebt sich die Frage, ob der Druck in den Phloemen dieses Blattes in einer bestimmten Relation zu seiner Flächengröße steht, da ihre Reproduktionsrate bzw. das Wachstum ihrer

Larven offenbar der Blattgröße bis zu einem gewissen Grade proportional ist. Es erscheint vorstellbar, daß eine größere assimilierende Fläche in den Phloemen der Hauptader eines Blattes — dem bevorzugten Sitz von *Aphis fabae* auf der Blattunterseite — höhere osmotische Drucke bedingt als eine kleine. Die Beobachtung Auclairs, daß *Acyrthosiphon* nach Hungerperioden zwar an anfälligen Erbsen eine gesteigerte Nahrungsaufnahmerate zeigt, nicht aber an resistenten, könnte dann damit eine gewisse Erklärung finden, daß eine solche Steigerung eben nur bei der höheren Kapazität der anfälligen Pflanzen möglich ist. In Zukunft muß bei ähnlichen Analysen der Ursachen antibiotischer Blattlausresistenz u.a. jedenfalls auch auf die Größe des „Einzugsgebietes" geachtet werden, die den sich entwickelnden Aphiden zur Verfügung steht.

Ob allerdings die Antibiosiseffekte der Ackerbohnenresistenz wirklich nur deshalb und allein von der Größe und Wüchsigkeit des Substrates abhängige Erscheinungen sind, weil sich nachweisen läßt, daß die erreichte Blattlausgröße eine Funktion der Substratfläche ist, erscheint fraglich. Ein so komplexes Kausalgeschehen wie das Wachstum kann zweifellos von völlig verschiedenen Faktoren beeinflußt und zu einander ähnlichen Ergebnissen gelenkt werden. So könnten bei *Acyrthosiphon pisum* die täglichen Hungerperioden auf anfälligen Sorten lediglich die gleichen Endeffekte wie die Entwicklung auf resistenten Sorten haben, d.h. sie könnten ähnliche Wirkungen erzeugen wie die Resistenz, sie gleichsam nur kopieren, ohne im Wirkungsmechanismus mit ihr identisch zu sein. Und ganz entsprechend wie Hunger bei *Acyrthosiphon pisum* könnte eine stark verminderte Fläche des anfälligen Substrates auf *Aphis fabae* wie der Antibiosiseffekt der resistenten Sorte wirken und doch in ganz andere Glieder des Kausalzusammenhanges eingreifen. Es wäre ebenso vorstellbar, daß Unterschiede in der individuellen Wüchsigkeit der Wirtspflanzen die eigentlichen Resistenzunterschiede der Sorten nur überdecken, indem sie sie entweder hemmen wie im Falle großblättriger Rastatter, oder fördern wie auf kleinblättrigen Schlanstedter Pflanzen, so daß sie sich dann nur scheinbar ausgleichen, in Wahrheit aber nur von gegensätzlich wirkenden Tendenzen in der Wüchsigkeit maskiert sind, die sich ihnen überlagern. Solange es nicht gelingt, Blattläuse auf künstlichen Substraten mit einer kontrollierbaren Diät zur Fortpflanzung zu bringen, werden sich über diese Alternativen kaum endgültige Entscheidungen treffen lassen.

Geht man freilich von der Vorstellung aus, daß die Aphidenentwicklung auf gutwüchsigen, anfälligen (Schlanstedter) Ackerbohnen das Normale und Optimale darstellt, dann sprechen die Hemmungen, die durch schwachwüchsige und kleinblättrige Pflanzen dieser Varietät in ähnlichem Maße wie durch vollwüchsige und großblättrige der resistenten hervorgerufen werden, und die weitere Verschärfung dieser Hemmung durch schwachwüchsige, resistente doch sehr dafür, daß die antibiotischen Effekte der resistenten Sorte einfache und relativ direkte Folgen einer gegenüber den anfälligen geminderten Baustoffwechselintensität (in der untersuchten Entwicklungsphase!) der Wirtspflanzen darstellen. Daß die Absterbe- bzw. Alterungsrate der Aphiden in der gleichen Weise zunimmt wie die Blattgröße sinkt, weist darauf hin, daß diese sich auch auf den Betriebsstoffwechsel der Bohnenläuse auswirkt.

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SUMMARY

RATE OF REPRODUCTION AND SIZE OF APHIS FABAE *ON YOUNG* VICIA FABA *PLANTS OF THE SAME AGE BUT DIFFERENT GROWTH ACTIVITY*

The biggest and smallest seeds of Schlanstedt (S or s) and Rastatt (R or r) field beans were selected in order to produce under otherwise identical conditions young plants of differing growth. When the third primary leaf pair unfolded the plants were decapitated so as to leave only one leaflet of the first pair. On such seedlings were determined the numbers and lengths of young *Aphis fabae* specimens borne by each young virginoparous aptera during the time from birth to maturity of her first-born daughter.

The young plants formed the series $S>s\geq R>r$ according to the size of the first leaf, and another series $S>R>s>r$ according to the cross sectional area of the stem. Quite similarly, the mean numbers and lengths of the first-born daughters were found to decrease as the leaf area of their host plants diminished, though the difference between R and s was not so great as between other members of the series. The mean length of the adult daughters was shown to be proportional to the mean leaf area available to each larva during postembryonic development. When R- and s-plants of identical growth activity were used, the antibiotic effects of their differing resistance against the bean aphids were no longer demonstrable between Schlanstedt and Rastatt field beans. Therefore one wonders whether the differing antibiotic resistance of these varieties should not be interpreted in terms of their differing growth intensities rather than as the result of some other quantitative or qualitative difference between them.

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THE INFLUENCE OF PHYSICAL FACTORS AND HOST PLANT ODOUR ON THE INDUCTION AND TERMINATION OF DISPERSAL FLIGHTS IN *SITONA CYLINDRICOLLIS* FAHR.

BY

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The reactions of *S. cylindricollis* to various weather factors were studied in the laboratory and in the field. The preferred temperature was dependent on the temperatures to which the weevils were adapted. Weevils emerging from hibernation had a lower temperature preference than weevils collected later in the field during the summer months. High humidity inactivated the weevils at all temperatures. Light did not change the reactions to temperature but high temperature reversed the negative reactions to light. The threshold ambient temperature for flight in the field in bright sunshine was 25° C. This implies a threshold body temperature of about 35° C which was found to be the threshold under laboratory conditions. A wind speed above 0.1 m/sec. decreased the flight activity and at 0.6 m/sec. flights ceased completely. Feeding and adaptation to high temperatures and light intensities increased the threshold for flight activity. Young weevils normally did not fly. The attractancy of colour and odour on flying insects was investigated with baited traps. *S. cylindricollis* did not show any reaction to colours. Coumarin proved to be a significant olfactory stimulus for terminating flights. Dispersal flights of *S. cylindricollis* occurred in both years mainly during a short period in May. Responses to temperature and light significantly regulated behaviour in the field. Later in the year the weevils do not often encounter flight-inducing high temperatures in their habitat.

The weevil, *Sitona cylindricollis* Fahr. is regarded as a limiting factor in the culture of sweet clover (*Melilotus officinalis* (L.) Lam. and *M. alba* Desv.) in the mid western region of Canada and the United States. The life history and some methods of control of this insect have been described by BIRD (1947, 1949) and MUNRO, LERAAS & NOSTDAHL (1949). THORSTEINSON (1960) reported a preliminary observation that the sweet clover weevil is attracted by the odour of coumarin, a characteristic constituent of its principal food plants.

Dispersal flights during May and June have been observed in the field (BIRD, 1947, 1949) and it is reasonable to assume that such flights are associated with initial infestations of fields of sweet clover. It would seem that these flights may in part be an adaptation to the annual or biennial habit of the food plants. A knowledge of the environmental factors most favourable to the initiation of dispersal flights would be useful for the timing of control measures. Confirmation of the role of food plant odour (coumarin) and information concerning the significance of photosensory reactions of the weevils would facilitate surveys of weevil populations in the field. The present paper is concerned with the study of light, temperature, humidity, and chemical influences on flight behaviour throughout the spring and summer.

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Most of the insects used in the following experiments were collected in the field each time immediately before the experiments were started. Some of the weevils were conditioned before use as explained.

BEHAVIOUR OF S. CYLINDRICOLLIS IN TEMPERATURE GRADIENTS

The temperature preferences for variously conditioned weevils were studied. The temperature gradient apparatus of HERTER (1934) was used with modifications. An aluminium plate, 60 cm length and 11 cm width was cooled at one end with frozen gelatine in plastic bags and heated at the other end with an electrically heated bar. The heat was regulated with a rheostat until a constant gradient from 10° to 35° C was obtained. The metal plate was covered with a transparent sheet of acrylic plastic supported at the edges by strips of thick cardboard to prevent the weevils from escaping. The temperature on the aluminium plate was measured by a telethermometer [1]). The weevils were always placed in the centre of the gradient area in groups of 50 after the gradient was stable. After 30 minutes the positions of the weevils were recorded. The activity of the weevils at different temperatures was observed.

The conditions in the temperature gradient were changed in the following ways: (1) the whole gradient was kept dark; (2) the temperature range from 10° to 24° C was illuminated; (3) the temperature range from 25° to 35° C was illuminated and (4) the whole gradient was illuminated with a photolamp (300 ft-c). All the experiments were repeated under two different conditions of humidity. In one series the gradient was kept relatively dry by placing filter paper over the area, in the other series the filter paper was moistened all through the gradient to provide a high humidity.

Weevils were investigated at various stages of maturity and in different states of conditioning as follows: Mature weevils recently emerged from the hibernating places at the end of April; weevils collected in August. Some of the weevils were conditioned for four days at 25° C or 4° C before being placed into the temperature gradient. Starved and fed and also light- and dark-adapted weevils were investigated.

Results and Discussion

Figure 1 shows that mature weevils from the hibernating places at the end of April had a mean preferred temperature of about 18° C. During June the preferred temperature increased to about 23° C. The difference between these means is significant ($p < 0.1$). The mean preferred temperature of young weevils in August was also about 23° C. The preferred temperature of mature weevils after conditioning for four days at 4° C was 17.5° C, resembling that of weevils from the hibernating places. After four days at 25° C the insects

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[1]) Model 44, Yellow Springs Instrument Co., Ind., Yellow Springs, Ohio.

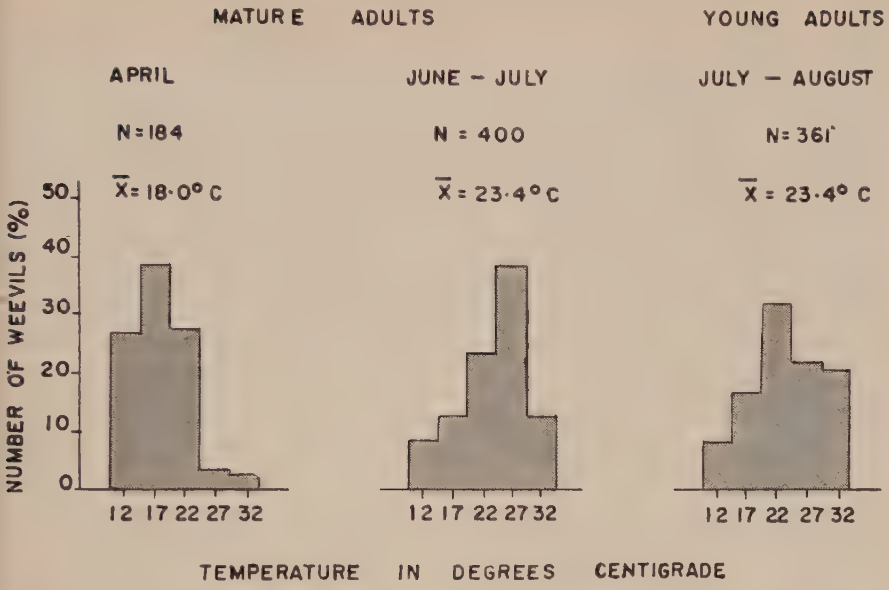

Figure 1. Distribution of *S. cylindricollis* in a temperature gradient at different times of the year.

preferred a temperature of about 22° C like weevils which were collected in the field during June.

All results mentioned so far were obtained in a dry temperature gradient. If the gradient was kept moist the weevils did not show any activity and most of them stayed in the position where they were placed. Neither light nor conditioning at different temperatures had any influence on the activity in the moist temperature gradient. Experiments done in a dry temperature gradient with a partly dark and partly light area showed that light does not change the temperature preference. The temperature preference of *S. cylindricollis* was not affected by feeding or starving. Only the pre-conditioning temperature influenced the temperature preference. The biological importance of this behaviour will be discussed in a later section.

REACTIONS OF S. CYLINDRICOLLIS TO LIGHT

The reactions towards light were tested in the laboratory in a dark and light chamber. One half of a plastic petri dish of 15 cm diameter was covered with black paper. The bottom of the other half was covered with white paper, the top being left transparent. The dish was placed in an incubator with a glass door, and maintained at 15°, 25° or 34° C. The incubator was illuminated from the outside either by diffuse daylight (40 ft-c) or by a photolamp (1000 ft-c). The dish was oriented to the light so that the border line between black and white displayed a sharp contrast in illumination. The weevils were always tested in groups of 30. The behaviour of the weevils was observed through 30 minutes when the positions of the weevils were recorded. Mature and young weevils previously adapted under various conditions were examined.

The justified objections of WELLINGTON (1960) against position recordings after a certain time, when the dependence of the photoreaction on the temperature is to be investigated, do not apply for this case for several reasons. The size of the dish was chosen with regard for the size and the locomotory speed of the insects so that they could reach light and shadow from all parts of the dish very quickly. There were no conspicuous accumulations of weevils observed at the border line between black and white but the weevils stayed both in the light as well as in the dark part of the dish always more at the junctions of the walls, responding evidently to a thigmotactic stimulus. This behaviour excluded, as single observations confirmed, a constant back and forward movement across the border line. We did not try to determine the exact temperature at which the photoreaction was reversed; observations indicated that the change is in any case dependent on the light intensity (Figure 2).

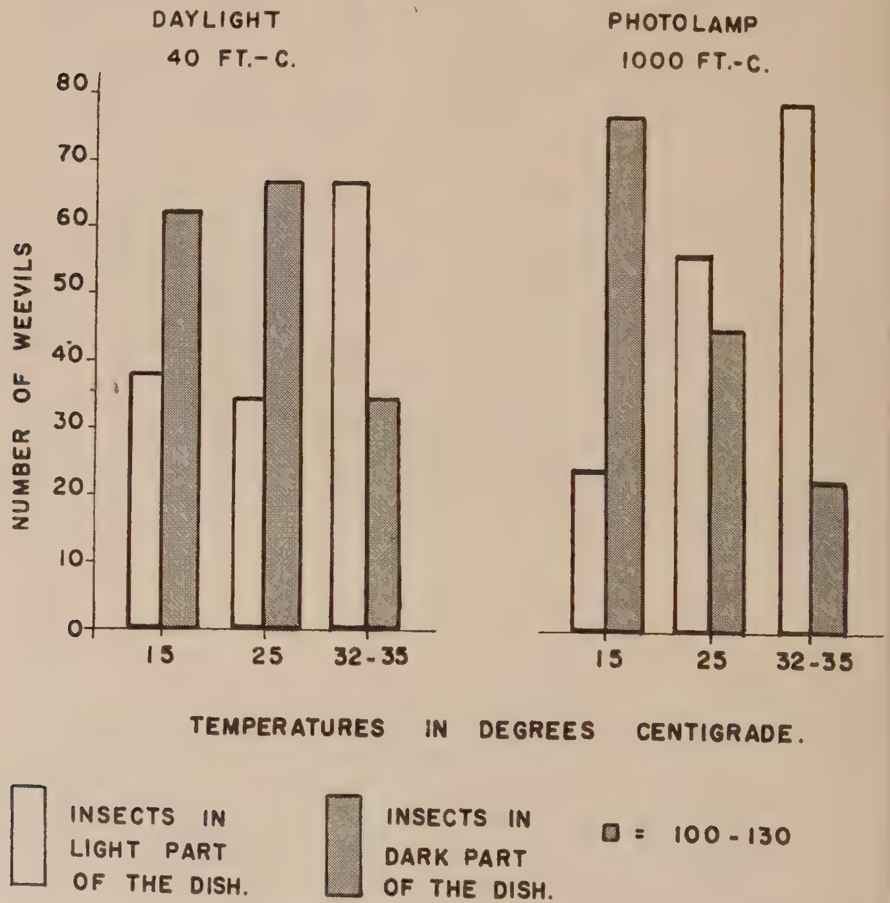

Figure 2. Reactions of *S. cylindricollis* to light at various temperatures. Number of weevils in %.

Results and Discussion

The reactions of the mature weevils are given in Figure 2. Young weevils reacted in a similar way. In an ambient temperature of 15° C most of the weevils stayed in the dark part of the chamber at both light intensities. At 25° C and a low light intensity (40 ft-c) the weevils still preferred darkness but at a light intensity of 1000 ft-c they showed a slight preference for the light part. Apparently the reversal temperature tends to decrease with increasing light intensity. At a temperature of 34° C most of the weevils were found in the illuminated part of the dish. This reaction was stronger in the higher light intensity. The differences between reactions at 15° C and 34° C are significant at the 5% level.

Young and mature weevils tried to fly at 35° C in the high light intensity. There was no difference in the reaction between fed and starved weevils. Mature weevils seemed to have stronger reactions to light than young weevils but the difference is not significant.

The fact that the reactions of *S. cylindricollis* to light are reversed by high temperature is important for flight induction and for protection of the weevils against high lethal temperatures near the ground. These results are discussed in a later section.

FACTORS AFFECTING THE FLIGHT ACTIVITY OF S. CYLINDRICOLLIS

Flights are important for the sweet clover weevil since dispersal mainly takes place by flying. It was observed previously that flights occur in spring (BIRD, 1949; MUNRO, 1949). Over a period of five years BIRD (1949) observed flights occurring in August only once. As the factors that induce these flights have not been studied, it was of interest to investigate whether they conform to those determined for the closely related insect, *S. lineatus* L. (HANS, 1959) and, for example, *Ceuthorrhynchus assimilis* (Payk.) (ANKERSMIT & NIEUKERKEN, 1954) and the red locust *Nomadacris septemfasciata* (Serville) (CHAPMAN, 1959). To this end, the flight inducing capacity of light, temperature, humidity and wind was investigated in the field and the laboratory.

Flight activity in the field

Weevils were collected in the beginning of May and placed in open cardboard cups on the ground. The temperature in the sun was measured with a telethermometer [1]), the light intensity with a photometer [2]) and the wind speed with a sensitive cup anemometer [3]). The conditions were recorded under which the weevils started to fly from the edge of the cup. Temperatures in the field could not be regulated independently of light intensity so the separate effects of these two factors had to be studied in the laboratory as described later.

The influence of wind speed over flight activity was considered only when other conditions were optimal, *viz.* temperature in the sun above 29° C and light intensity above 5000 ft-c. The results are given in Figure 3.

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[1]) Model 44, Yellow Springs Instruments Co. Inc., Yellow Springs, Ohio.

[2]) Model 756 with Quartz Filter, Weston Electrical Instrument Corp., Newark, N.J.

[3]) Model 1420, Fa. Lambrecht, Goettingen, Germany.

Flight activity in the laboratory

The insects were placed in a paper cup into an incubator with a glass door at temperatures of 25°, 30°, 35° and 40° C. From outside the incubator was illuminated either with a photolamp (1000 ft-c) or by daylight (40 ft-c). The weevils were watched continuously for 10 minutes and the numbers of flights or flight attempts were recorded. At each temperature and light intensity at least 100 insects were tested in groups of five. Fed and starved, mature and young weevils were studied. They were also investigated after being adapted for four days at 25° C or 4° C; or two days to darkness or light at 25° C.

In another experiment the influence of the temperature of the substratum was observed. A hot plate was surrounded by a paper cylinder 15 cm high, and heated to about 35° C. The weevils were placed on the plate and illuminated with a photolamp (1000 ft-c). The air temperature was 25° C. Again the insects were tested in groups of five at one time, in total 95 weevils.

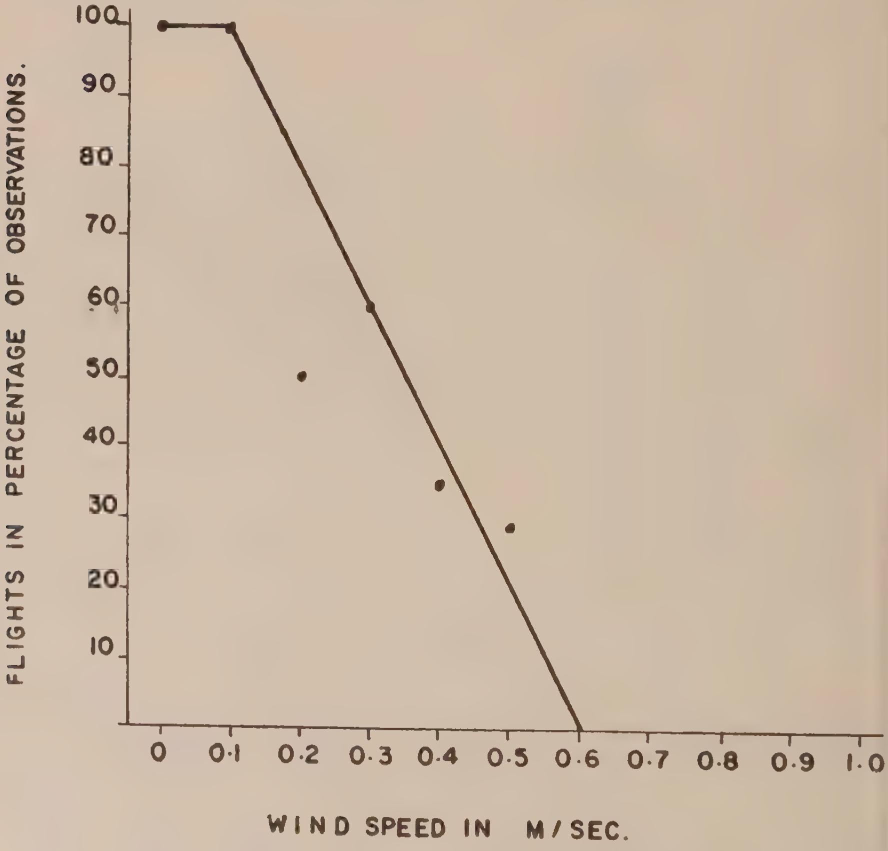

Figure 3. Flight activity of *S. cylindricollis* in relation to wind speed.

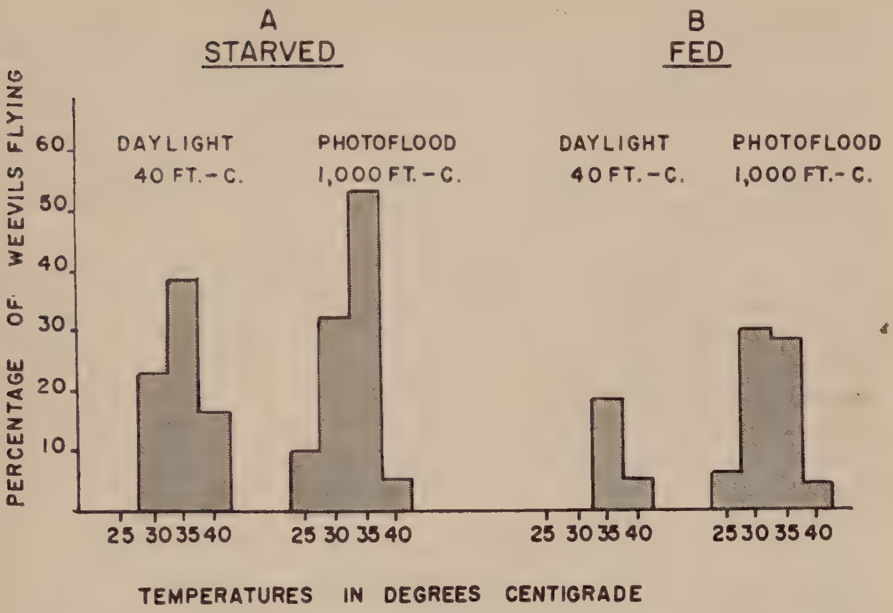

Figure 4. Flight activity of *S. cylindricollis* in relation to temperature, light intensity and nutritional state.

Results and Discussion

The flight preparations of *S. cylindricollis* are characteristic. First the weevils react positively to light and climb to the highest point they can reach on a plant (in our experiments the rim of the beaker). If flight conditions are favourable, they raise their front legs and move them up and down very quickly. After a short while the elytra are opened and the wings unfolded. If the conditions are still suitable the weevils take off in flight immediately after these preparations. Otherwise they draw the wings back under the elytra and walk some distance. A return of favourable conditions induces the flight preparations again. Figure 3 shows the influence of wind speed on flight activity. At wind speeds up to 0.1 m/sec., all weevils were flying but at speeds from 0.1 to 0.6 m/sec., the flights decreased and normally ceased altogether at higher wind speeds. Sometimes very high temperatures induced flight attempts at high wind speeds but these attempts were seldom successful.

The highest flight activity was found at 35° C (Figure 4 A). In the laboratory more weevils were induced to fly at high light intensity than at low. This was true at all temperatures between 25° and 35° C, but at 40° C more weevils flew at the lower light intensity. At 25° C, no flight attempts were induced at low illumination. The temperature at which most weevils flew was the same at both light intensities. These results indicated that light interacts with temperature in the induction of flight. At 40° C, the temperature is supra-optimal if light intensity is high. Fewer flights are performed apparently because most of the weevils are too excited to carry out coordinated movements. These inhibiting high temperatures were not observed in the field.

In the experiments with the hot plate the weevils reacted to the high temperature first by running, then by flying. As soon as they reached the paper wall and lost contact with the plate they did not make any further attempts to fly. Possibly, tarsal receptors mediated this response to high temperature of the substratum.

A comparison of Figure 4B with 4A shows that inanition favours responsiveness to flight stimuli. In fed weevils the threshold for flight induction is much higher than in starved weevils. The same results were obtained for *S. lineatus* (HANS, 1959) and the red locust (CHAPMAN, 1959).

In further experiments the influence of previous adaptation to various temperatures and lighting on flight activity was investigated. Weevils which were kept cold (4° C) for four days started to fly in 56% of observations, weevils kept warm (25° C) only flew in 18% of the trials. If the insects were adapted to light as well as to a 25° C temperature, no flights occurred.

Weevils, collected in the field at the end of May, were placed in flats with sweet clover in the greenhouse and covered with screened cages. The air temperature in the sun was 38° C and the light intensity 4000 ft-c. One flat was planted with little seedlings of sweet clover, another with sweet clover plants 20 cm high. In the flat with the seedlings the weevils tried to hide in the soil. When the soil proved too hard flights and flight attempts were performed repeatedly until the weevils were exhausted. The insects were very excited and on the following day many dead ones were found in the flat. In the cage with the taller plants the weevils could hide in the shade of the plants. They moved around at normal speed and no flights were observed. No dead weevils were found the next day. Although individual young weevils were occasionally seen flying under conditions of extreme temperature in the laboratory in August, systematic experiments in the field and laboratory failed to confirm these observations.

In general the experiments show that a sufficiently high temperature (about 35° C) is a governing factor for flight induction. The same observations were made for *S. lineatus* (HANS, 1959) and the red locust (CHAPMAN, 1959). For both *Sitona* species the optimal temperature for flight is much above the preferred temperature as estimated in a previous section. A high light intensity enhances the flight response. In the field this optimal temperature occurs only on days with bright sunshine.

The extensive literature on body temperatures in insects exposed to the sun has been recently reviewed by EDNEY (1957). From investigations on various insects (KRÜGER, 1931; KRÜGER & DUSPIVA, 1933; DIGBY, 1955 and others) a body temperature of about 10° C in excess of ambient temperature can be assumed for *S. cylindricollis* in bright sunshine. This would explain why the weevils flew in the field at an air temperature of 25° C in bright sunshine while they only flew readily in the laboratory at an air temperature of 35° C. One of the factors which can influence the temperature excess is the wind speed (KRÜGER & DUSPIVA, 1933; DIGBY, 1955; CHURCH, 1960). Even a slight wind in excess of 0.1 m/sec. decreases the body temperature below the optimum for flight.

The lower response of fed weevils to flight stimuli may be caused either by a general decrease in activity after feeding as is described by BARTON BROWNE & EVANS (1960) for the locomotor activity of the blowfly. Alternatively, the higher water content of the weevils associated with food in-take may increase transpiration at high temperatures, and decrease the temperature to a level below the optimum for flight. The ability of insects to decrease the body temperature in the sun by increased transpiration was investigated by several authors (reviewed by EDNEY, 1957). Arthropods vary considerably in this respect. Observations of flight induction at different humidities showed that the weevils did not even try to fly at high humidity. It will be recalled that flight is always preceded by climbing or walking and that high humidity inhibits ambulation. It would seem to follow that flight is not likely to occur at high humidity.

CHAPMAN (1959) observed that rapid increases in temperature induce more flights by the red locust than a gradual increase allowing adaption of the weevils. This is in harmony with our results with warm and cold adapted weevils mentioned previously. In our experience rapid and large changes in light intensity are likewise more stimulating for flight than slower changes, suggesting an adaption effect. The interpretation of these results in terms of the field behaviour of the weevils is discussed in a later section.

INFLUENCE OF COLOUR AND ODOUR ON THE TERMINATION OF FLIGHT

The influence of colour and coumarin odour (THORSTEINSON, 1960) on the landing of *S. cylindricollis* was confirmed by the use of traps modified after MOERICKE (1952). This study was made in a summer fallow field in 1959 and 1960 at least one mile distant from sweet clover fields. Plastic dishes, 30 cm diameter and 7 cm high, coloured white, grey, black, yellow, orange, red, blue, green and transparent were used, each colour in eight replicates. The dishes were recessed a little into the soil to keep them from being blown away by the wind. The distribution of the colours in blocks was randomized. Each block consisted of three rows, six feet apart with three dishes, two feet apart. In alternating blocks little dishes each containing 200 mg coumarin were placed in the traps. Colourless and odourless mineral oil was used as trapping fluid. Oil and coumarin were replenished from time to time as necessary, for example after heavy rainfall. At the same time the blocks with and without coumarin were interchanged. The traps were inspected and the number of captured weevils was recorded daily at 9 a.m. In 1959 the traps were not set out until June 1, but in 1960 the observations were started on May 8. In both years the traps stayed out in the field until the middle of September.

Results and Discussion

In 1959, only 29 weevils were caught all through the summer since the flight season was almost finished at the beginning of June. No influence of any colour or coumarin was evident in this small number of captures. The results obtained in 1960 are shown in Figure 5. The main time for flights was in May. After June 1, only occasional flights occurred. No young weevils were captured in 1959 nor in 1960.

The traps with coumarin caught significantly more weevils than the unbaited traps ($p < 0.5$). Among the various colours there was no difference in the numbers of weevils caught in 1960 and it appears that *S. cylindricollis* does not

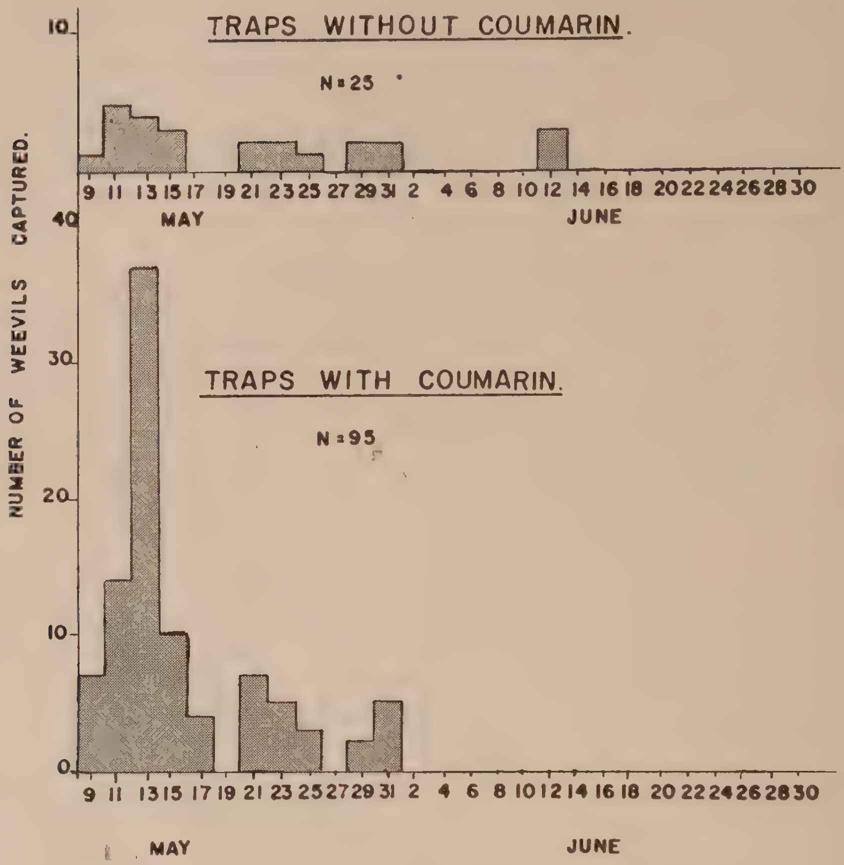

Figure 5. Attractancy of coumarin for flying *S. cylindricollis.*

react to colours on the ground during the dispersal flights. This observation agrees with that of Hans (1959) for *S. lineatus.* It appears clear that coumarin odour is a significant food plant stimulus that induces termination of the dispersal flight. It is of interest that a sweet clover variety (Coumino) has been developed which contains no coumarin. On the basis of our data one might expect that fields of this variety should be less likely to become infested during the spring flights than fields of clover containing coumarin. No confirmation of this possibility is as yet available.

BEHAVIOUR OF S. CYLINDRICOLLIS IN THE FIELD IN SPRING AND SUMMER

On the basis of the studies of the effects of individual factors we will try to outline a picture of the behaviour of *S. cylindricollis* in the field through spring and summer. The factors inducing and limiting dispersal flights will be emphasized. From the end of April to the beginning of May the weevils become active in the hibernating places as soon as the temperature rises. The sweet clover plants also start growing but at this time they have developed only a few small leaves which do not provide protection to the weevils against the heat of the sun. A combination of several factors induces flight in the weevils at this time of the year. The soil is heated up by the sun much quicker and to a higher temperature without the shade of the foliage. The weevils, being adapted to low temperatures, are more ready to fly than later after being adapted to warmer temperatures. Besides, the daily temperature changes are greater at this time and the weevils coming from hibernating quarters are starved. All these factors increase the readiness for flight.

Since the reaction to light is reversed to positive at high temperature the weevils come out of the hiding places as soon as it gets warm enough for a positive photoreaction and start flying. The range and duration of flight of *S. cylindricollis* is unknown but ultimately many of the dispersing weevils succeed in finding fields of sweet clover in which to land. Presumably, the probability of this event is increased by the influence of coumarin on termination of the flights. After landing they may take off again several times. This take-off and landing behaviour lasts until the plants are grown high enough to cover the soil with shade and until the weevils are adapted to warmer temperatures. They feed on the plants and thereby become less responsive to flight stimuli. In the shade of the foliage no great temperature changes take place in the habitat of the insects. All these factors raise the threshold for flight activity slowly during the month of May.

The captures in 1959 and 1960 demonstrate that after the beginning of June flights rarely occur. This might be expected from the fact that in a lush crop of sweet clover the temperatures rarely reach 34° C.

In a lush crop of sweet clover the weevils are to be found during the day at the base of the plant at the junction of the stem and the soil surface or, under drier conditions, below a thin crust of dry surface soil. At night the weevils may be found on the upper leaves of the plants and nearly all feeding is done at this time. If feeding weevils at night are illuminated with a flashlight they immediately hide under the leaves or drop to the ground. This suggests that perhaps at dawn the increase in illumination is responsible for the return to the soil. The internal moisture derived from the food, together with soil moisture if present, negative phototaxis and probably positive thigmotaxis combine to keep the weevils in this situation during the day. If dew forms the high humidity inhibits the nocturnal ascent to the leaves of the food plants. Otherwise the weevils become active possibly through the influence of inanition and they climb up the plants. Since nearly all of them reach the top leaves negative geotaxis seems to be involved. This phase of the behaviour of *S. cylindricollis* conforms closely to that of *S. lineatus* which was investigated in greater detail (HANS, 1959).

When the young weevils emerge from the soil in July or August they are adapted to high temperature and have a high threshold of response to flight stimuli. If the sweet clover is cut, the weevils migrate by ambulation and often enter a nearby sweet clover field. In both years it was observed that only young sweet clover fields closest to the old field were attacked, mainly at the edges, while more distant fields in the neighbourhood remained uninfested.

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ZUSAMMENFASSUNG

DER EINFLUSS PHYSIKALISCHER FAKTOREN UND DES WIRTSPFLANZENDUFTES AUF DIE AUSLÖSUNG UND BEENDIGUNG DER AUSBREITUNGSFLÜGE VON SITONA CYLINDRICOLLIS *FAHR.*

Das Verhalten von *S. cylindricollis* gegenüber einzelnen Wetterfaktoren wurde im Laboratorium und im Freiland untersucht. Die Vorzugstemperatur war abhängig von der Temperatur, an welche die Käfer adaptiert waren. Käfer aus den Winterquartieren hatten eine niedrigere Vorzugstemperatur als Käfer, welche während der Sommermonate im Freiland gesammelt wurden.

Hohe Luftfeuchtigkeit inaktivierte die Käfer in allen untersuchten Temperaturstufen. Licht beeinflusste die Reaktionen gegenüber Temperatur nicht, aber hohe Temperatur kehrte das photonegative Verhalten um. Zur Flugauslösung war der Schwellenwert in der Umgebungstemperatur 25° C im Freiland in hellem Sonnenschein. Dies bedeutet einen Schwellenwert in der Körpertemperatur von 35° C, wie in Laboratoriumsversuchen gefunden wurde. Eine Windgeschwindigkeit von über 0.1 m/sec. beeinflusste die Flugaktivität bereits, und bei 0.6 m/sec. hörten die Flüge ganz auf. Nahrungsaufnahme und Adaptation an hohe Temperaturen erhöhten die Reizschwelle für Flugaktivität. Jungkäfer flogen normalerweise nicht.

Das Anziehungsvermögen von Farben und Geruch auf fliegende Käfer wurde in farbigen Köder-Fallen untersucht. *S. cylindricollis* zeigte keine Reaktionen gegenüber Farben. Cumarin erwies sich als wirkungsvoller Geruchsreiz um Flüge zu beenden. Verbreitungsflüge fanden in beiden Jahren hauptsächlich in einer kurzen Periode im Mai statt. Reaktionen gegenüber Licht und Temperatur regulieren nachweislich das Verhalten des Süsskleekäfers im Freiland. Später im Jahr sind die Käfer in ihrem Habitat flugauslösenden hohen Temperaturen meistens nicht mehr ausgesetzt.

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PREVENTION AND ARTIFICIAL INDUCTION OF IMAGINAL DIAPAUSE IN *COCCINELLA SEPTEMPUNCTATA* L. (COL.: COCCINELLIDAE)

BY

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Excess food, long photoperiod, and high temperature prevents diapause in *Coccinella septempunctata* L. after the beetles had been reared through several generations under these conditions. These is an interaction between photoperiod and temperature in induction of diapause, photoperiod being the more important factor. A long photoperiod inhibits diapause even at a low temperature. The proportion of diapausing females in later generations varies inversely with the temperature if the photoperiod is short. Which factor induces diapause in adults of the first generation is not yet known.

The physiological condition of beetles artificially induced to enter diapause is apparently similar to that of beetles in diapause in the field.

Diapause in *C. septempunctata* occurs at a time when environmental factors apparently still favour development and activity. Beetles in diapause have a large fat body and ovaries with not yet differentiated germaria (HODEK & ČERKASOV, in litt.).

The second phase of imaginal dormancy is a quiescent period during which the resumption of activity and development can be evoked by favourable conditions (HODEK, 1960).

DOBRZHANSKY (1922) states that the incidence of diapause in coccinellids is not affected by environmental conditions. JAKHONTOV (1940) reared coccinellids under controlled conditions, which unfortunately he fails to describe, and he succeeded only in increasing slightly the number of generations occurring in the field. Therefore the prevention and induction of diapause in coccinellids under controlled conditions merits further investigation.

MATERIALS AND METHODS

Beetles collected in the field after they emerged from hibernation (Generation 0) were reared in the laboratory under crowded conditions in cylindrical glass or plexite cages of various sizes, each covered with a silon lid. One or twice daily, plant material infested with aphids was placed on a piece of zigzag folded filter paper in each cage. Eggs which were laid by the coccinellids on this filter paper were also collected daily. Larvae emerging from these eggs were transferred to another cage and in this way the generations were kept separate. Some larvae and adults were kept singly in 11 cm × 14 cm vials covered with silon and containing a piece of filter paper. The number of eggs laid and the mortality in each cage were recorded daily.

The proportion of active beetles in each generation was determined by dissecting the adult females. Twenty-day-old females with ovaries consisting of undifferentiated germaria only were recorded as being in diapause. No apparent difference is found between testes of active and diapausing males.

Cultures were started in five successive seasons, the first in 1956, the fifth in 1960. At the end of each season, after about ten generations were bred the cultures were brought to an end by increasing mortality (see also Table VIII).

The adults used to start the first culture were kept under nearly natural conditions of temperature and daylength. Cages containing the second and later generations of the first culture, and all generations of the other cultures, were kept in a constant temperature room maintained at between 22° C and 28° C, and under artificial fluorescent lighting kept on for a period of 16 hours at first, and after October 1958, 18 hours a day. The first three generations of the first culture were fed on the aphids, *Aphis fabae* Scop., *Uromelan aeneus* H.R.L., *Anoecia corni* F., and *Pergandeida craccae* L., collected in the field. Later generations of the first culture, and the larvae and adults of the other four cultures, were fed on *Pergandeida craccivora* Koch, the parthenogenetic viviparous generations of which were reared on *Vicia faba* L. under the same conditions of daylength and temperature as the coccinellids.

The number of generations which appeared in the cultures was slightly lower than that which is theoretically possible. In the first culture 9 generations completed their development in a year; in the second, only 8; in the third, 9 generations were reared in 11 months; and in the fourth, the theoretical number was obtained: one generation a month.

In the first culture a constant excess of food was maintained by providing aphids twice a day. Fewer aphids were provided to the second and third cultures, where intermittant shortages of food occurred. In the fourth culture food was rather more plentiful.

The first experiment on induction of diapause was carried out in a light proof cage kept in a room maintained at 23° C. The cage was internally illuminated by a small tungsten filament lamp. Heat from the lamp raised the temperature within the cage to 28° C. Incubators, similarly illuminated, and kept in a cellar room where the temperature varied from 10° C in winter to 15—17° C in summer, were used for the other experiments. Heat from the lamps did not raise the temperature in the air-jacketed incubators, but various controlled ranges of temperature could be obtained in the water-jacketed incubators by using lamps of different wattages.

The percentage glucose content of adult beetles was determined by the anthrone method (CARROL, LONGLEY & ROE, 1956) after hydrolysis of desiccated specimens with potassium hydroxide and precipitation with alcohol (WALAAS & WALAAS, 1950). The intensity of colour was measured on a Lange's colorimeter. By multiplying by the coefficient 0.9 the glucose values were then converted into glycogen values. The percentage fat content was determined gravimetrically after extraction of the homogenised coccinellids with alcohol-ether and purification with ether. A more detailed description of the method will be given elsewhere (HODĚK & ČERKASOV, in litt.).

The temperature ranges used in the experiments are given in the shortened forms, 19—22.5° C (D)/22—25° C (L). In the course of the experiment the temperature may increase gradually from the lower to the greater value (→), or alternate in the given range (—). The temperature range differed in the dark (D) and light periods (L).

RESULTS

Situation under field conditions

Early in July in 1959 and 1960, 202 adult *C. septempunctata* females, collected in sugar-beet fields in Central Bohemia, were dissected. Only newly emerged adults with orange elytra were taken, so that adults which had already overwintered were excluded.

TABLE I

Dissection of females collected in the field

Instar collected	date collected	date dissected	number dissected	percentage diapausing females*
prepupae or pupae	19.6.59	13.7.59	19	90.5
adults	6.7.59	16.7.59	59	93.2
adults	17.7.59	18.7.59	79	92.4
adults	13.—15.7.60	21.7.60	45	84.4

*) females with ovaries consisting of undifferentiated germaria only.

The beetles were collected when day length was longest and in both years it was warm and there was an abundance of aphids in the sugar-beet fields. Nevertheless, in all the samples collected the majority of the females (84.4—93.2%) had ovaries with undifferentiated germaria only (Table I). Some of the beetles were kept for ten days in warm field conditions but the ovaries remained unchanged.

TABLE II

The proportion of diapausing females in each generation of culture I of C. septempunctata *(1956)*

generation	date of emergence of adults	age of adults at dissection (days)	number dissected	percentage diapausing females*
1	3. 7.— ?	?—54	22	86.4
2	10. 8— ?	?—99	11	63.6
3	30. 9.— 7.10.	38—45	9	11.1
4	6.11.—10.11.	36—40	9	0
5	9.12.— ?	?—44	17	0
6	21. 1.—23. 1.	58—60	10	0
7	3. 3.—11. 3.	50—54	10	0
10	25. 7.—21. 8.	28—55	14	0
12	27.10.—11.11.	28—43	10	10

* See note under Table I

Continuous cultures

In the first culture the proportion of diapausing females gradually decreased over the first three generations and in the following six generations no diapausing females were recorded (Table II). A certain variable proportion of diapausing beetles was recorded in the later generations of the second, third and fourth cultures (Tables III, IV & V). The differences in the availability of food between the cultures may be responsible for this difference between cultures. Aphids were supplied in constant excess to the ladybirds in the first culture whereas in all the other cultures fewer aphids were provided.

TABLE III

The proportion of diapausing females in each generation of culture II of C. septempunctata *(1957)*

generation	date of emergence of adults	age of adults at dissection (days)	number dissected	percentage diapausing females*
1	12. 7.—12. 8.57	37—68	27	63.0
3	26.10.—13.11.57	42—60	14	14.3
4	3.12.—12.12.57	37—46	19	15.8
5	20. 2.— 7. 3.58	46—61	30	6.7
6	15. 4.—27. 4.58	38—50	18	16.7
8	18. 6.—30. 6.58	56—68	11	45.4
9 A	30. 7.—24. 8.58	22—47	29	10.3
B		36—61	29	10.3

* See note Table I

TABLE IV

The proportion of diapausing females in each generation of culture III of C. septempunctata *(1958)*

generation	replicate	date of emergence of adults	age of adults at dissection (days)	number dissected	percentage diapausing females*
1		25. 6.— 9. 7.58		18	66.7
2	A	7. 8.—29. 8.58	17—39	10	20.0
2	B	7. 8.— 4. 9.58	35—63	14	50.0
2				24	37.5
3		11. 9.— 9.10.58	27—49	11	18.2
4	A	20.10.— 8.11.58	33—52	23	47.8
4	B	1.11.—19.11.58	40—58	16	12.5
4				39	33.3
5		24.12.—30.12.58	30—36	16	6.3
6	A	20. 1.—25. 1.59	40—45	19	31.6
6	B	26. 1.—29. 1.59	36—39	16	6.3
6	C	29. 1.—11. 2.59	23—36	21	4.8
6				56	14.3
7		23. 2.— 1. 3.59	33—39	13	7.7
8	A	31. 3.—13. 4.59	42—55	11	9.1
8	B	17. 4.—20. 4.59	37—40	13	7.7
8				24	8.3
9	A	22. 4.— 4. 5.59	25—37	9	11.1
9	B	13. 5.—17. 5.59	22—26	12	0
9				21	4.8

* See note Table I

TABLE V

The proportion of diapausing females in each generation of culture IV of C. septempunctata *(1959)*

generation	replicate	date of emergence of adults	age of adults at dissection (days)	number dissected	percentage diapausing females*
1	A	16. 6.—18.6.	23—25	12	33.3
	B	20. 6.—24.6.	17—21	10	30.0
				22	31.8
2	A	14. 7.—19.7.	43—49	4	0
	B	22. 7.—24.7.	39—41	2	0
	C	26. 7.—29.7.	34—37	5	20.0
				11	9.1
5	A	26.11.—29.11.	21—24	11	9.1
	C	4.12.—10.12.	27—33	10	0
	D	8.12.—11.12.	27—30	11	0
	E	11.12.—17.12.	20—26	5	0
	F	16.12.—23.12.	14—21	7	14.3
	G	22.12.—26.12.	11—15	6	0
				50	4,0
6	A	1.12.— 9.12.	10—19	11	9.1
	B	23. 1.—30.1.	18—25	11	0
				22	4.5
8	A	29. 1.— 2.2.	37—41	6	16.6
	B	8. 2.—16.2.	23—31	7	14.3
	D	26. 2.— 1.3.	15—19	23	0
				36	5.5
9		22. 2.— 1.3.	15—23	10	10.0

* See note Table I

A relatively high percentage of diapausing females occurred in the first generation of all four cultures, and also in the first generation reared in 1960 (Table VI & VII).

TABLE VI

The proportion of diapausing females in the first generation of culture Va of C. septempunctata *(1960)*

replicate	date of emergence of adults	age of adults at dissection (days)	number dissected	percentage diapausing females*
A	7.3.— 8.3.	30—31	13	46.2
B	8.3—10.3.	28—30	13	100.0
C	10.3.—12.3.	26—28	18	88.9
D	11.3.—14.3.	28—31	7	85.7
F	13.3.—14.3.	28—29	13	84.6
G	15.3.—16.3.	31—32	10	70.0
H	17.3.—19.3.	28—30	14	92.9
I	18.3.—19.3.	28—29	20	85.0
J	19.3—20.3.	32—33	21	76.2
K	25.3.—27.3.	25—27	8	75.0
			137	81.0

* See note Table I

TABLE VII

The proportion of diapausing females recorded in the first generation of C. septempunctata *at different temperature ranges and with 16—19 hours of light each day*

year	cultures	replicate	temperature	date of emergence of adults	age of adults at dissection (days)	number dissected	percentage diapausing females*
1956	I			3.7.— ?	?—54	22	86.4
1957	II		ca 25° C const. (with slight changes)	12.7 12.8.	37—68	27	63.0
1958	III			25.6.— 9.7.		18	66.7
1959	IV			16.6.—24.6.	17—25	22	31.8
1960	V a**			7.3.—27.3.	25—33	137	81.0
1960	V b	1 A	D : 22 → 25/L : 28 → 31° C	27.6.— 1.7.	24—28	13	38.5
		1 B		1.7.— 4.7.	21—24	14	50.0
		1 C		3.7.— 6.7.	22—25	12	66.6
						39	51.3
		2 A	D : 19 → 22.5/L : 22 → 25° C	10.7.—12.7.	20—22	16	0
		2 B		16.7.—17.7.	15—16	17	29.4
						33	15.2
		3 A	D : 25 → 26/L : 27 → 29.5° C	17.7—22.7.	17—22	18	72.2
		3 B		23.7.—27.7.	12—16	13	53.8
						31	64.5

* See note under Table I

** See also Table VI.

In the first culture a high mortality occurred in the 11th and 12th generations, especially in the 12th. Also a striking decrease in fecundity of the female beetles and longevity of the males was observed when adults from the second culture were reared singly in vials from the third to the sixth generation (Table VIII).

TABLE VIII

Fecundity and longevity in generations three, four and six in the second culture of C. septempunctata

generation	No. of days on which oviposition occurred	average longevity (days)		number	
		females	males	females	males
3	20.6	59.4	71.3	26	27
4	13.3	62.9	60.5	29	30
6	7.7	47.6	46.9	23	30

Although no precise general conclusions can be drawn from a comparison of the proportion of diapausing females in the different experiments, as not all conditions were constant, e.g., the number of specimens used to start a culture, locality from which the beetles were collected, age of females at the time of oviposition and time of dissection, number of specimens bred and dissected, nevertheless certain facts emerge:

1. Diapausing females are recorded in the first generation of all the cultures even under conditions otherwise favourable for the prevention of diapause.
2. In the later generations of the first culture kept under optimal conditions of photoperiod, temperature and food supply diapause does not occur, but any deviation from the optimal conditions, as in cultures 2, 3 and 4, results in some beetles entering diapause.
3. After about 10 generations under constant conditions, the vitality of the insects decreases and there is high mortality.
4. Under controlled conditions it is possible to maintain a laboratory culture of ladybirds producing 10—12 generations in a year providing the culture is supplemented every spring by the addition of beetles collected in the field after they have emerged from hibernation. The only difficulty in maintaining a culture is procuring a plentiful supply of aphids especially during the unfavourable part of the year. Perhaps an artificial diet, using dried aphids could be used for this purpose (SZUMKOWSKI, 1952; SMIRNOFF, 1958).

Artificial Induction of Diapause

Throughout their larval and early imaginal development a number of individuals of *C. septempunctata,* taken from the later generations of the cultures, were subjected to various combinations of photoperiod and temperature ranges. The results are recorded in Table IX. Temperature obviously influences the proportion of diapausing females at a given photoperiod.

At relatively low temperatures a short photoperiod induces a high proportion of females to enter diapause. High temperatures, even when combined with the shortest photoperiod used, markedly inhibit the induction of diapause. A long

TABLE IX

The proportion of females of C. septempunctata *entering diapause under different conditions of temperature and daylength*

source of material		age of larvae at beginning of experiment (days)	temperature range (° C)	photoperiod (hours per day)	age of adults at dissection (days)	number dissected	diapausing females %
culture	generation						
I.	7.	3	17—18(D)/20—22(L)	12	42—51	15	93.9
I.	7.	pupae	17—18(D)/20—22(L)	12	56—62	15	86.6
I.	9.	5—8	17—18(D)/20—21(L)	12	24—41	30	86.7
I.	9.	5—7	22 (21.5—22.5)	12	33—35	10	70
II.	4.	4—5	22 (21 —22.5)	8	24—34	42	59.5
I.	9.	3—7	25 (24.5—25.5)	12	30—33	40	32.5
I.	6.	1	24—25(D)/27—28(L)	8	24—26	20	10
III.	4. 5.	2—3	18 (17.5—18.5)	19	26—43	24	12.5
II.	4.	6—7	18—19(D)/20—21(L)	16	35—46	6	0

photoperiod inhibits diapause at all temperatures used, even at such a low temperature that larval development scarcely proceeded.

In experiment I/7 where pupae instead of young larvae were transferred from the culture to experimental conditions, no difference was found between the adults obtained from these pupae and the adults from larvae kept under similar experimental conditions from an early age.

Preliminary investigations were made of the effect of physical factors on the termination of artificially induced diapause. By keeping females in diapause under conditions of 12 hours of light per day at high temperatures, they gradually came out of diapause. After 68—70 days 64% had emerged from diapause. In experiment I/9b none remained in diapause after 80—97 days, irrespective of the experimental temperature used (Table X). Thus artificially induced diapause does not seem to need low temperature for its termination.

TABLE X

Results of keeping diapausing female beetles of C. septempunctata *for varying periods at different temperatures (12 hr. photoperiod)*

	Experiment I/9 a		Experiment I/9 b		
time of treatment in days*	33—35(I)	68—70(I)	24—41(II)	59—76(II) + 21(IV)	24—41(II) + 35(III) + 21(IV)
number of females dissected	10	39	30	7	11
per cent of diapausing females	70	36	86.7	0	0

*Temperature I . . . . 22° C const.
II . . . . 17—18° C (D)/20—21° C (L)
III . . . . 4—5° C const.
IV . . . . 25° const.

Comparison of adults naturally and artificially induced to enter diapause

Dissections revealed no difference in the ovaries or fat body between adults in diapause either collected in the field or reared in the laboratory.

Chemical analysis was carried out on adult males and females in diapause collected in early October from a hibernating site (Raná-hill, near Louny, Northern Bohemia) and on diapausing and normally active females reared in the laboratory. Laboratory reared females were first dissected to ensure that they were in diapause, or active, and were then analysed together with the alcohol in which they were dissected.

The proportion of glycogen is practically the same in beetles naturally and artificially induced to enter diapause. The fat content is one fifth lower in the laboratory reared beetles (Table XI). Active females contain half the quantity of glycogen and less than half (43.9%) the quantity of fat that females in diapause contain. Hence the physiological state of the adults in diapause, whether taken in the field or reared in the laboratory, can be considered equivalent in this respect.

TABLE XI

The proportion of fat and glycogen in diapausing and active adults of C. septempunctata

	proportion of fat (mg of lipids in mg of fresh weight)		number of specimens	proportion of glycogen (γ of glycogen in mg of fresh weight)		number of specimens
	♀♀	♂♂		♀♀	♂♂	
Adults naturally induced to enter diapause	0.216	0.190	7	8.64	9.16	3
	0.223	0.219	7	6.00	7.00	3
	0.204	0.212	7	8.40	10.80	3
	0.177	0.219	7	7.05	8.76	3
	0.205	0.210	28	7.55	8.94	12
Adults artificially induced to enter diapause	0.144		7	8.00		2
	1.184		6	6.74		2
	0.164		13	7.36		4
Active adults (reared in laboratory)	0.073		9	3.78		2
	0.070		8	3.60		2
	0.072		17	3.69		4

DISCUSSION

Several normally univoltine temperate zone insects, such as *Stenocranus minutus* Fabr. (Müller, 1957), are potentially polyvoltine; i.e. they continue to reproduce as long as environmental conditions remain favourable. In populations of *Coccinella septempunctata* in Czechoslovakia, the situation is not so clear.

Dobrzhansky (1922) believes that induction of diapause in *Coccinella septempunctata* L. and other coccinellids is quite independent of environmental conditions and is directed by internal stimuli. But the present results indicate an evident dependence of the induction of diapause on environmental conditions. The precise relationship between environment and the induction of diapause is, however, not fully understood.

Even when the first generation obtained from overwintering adults of *C. septempunctata* has developed under apparently favourable conditions both in the field as well as in laboratory cultures, a very high proportion of the females entered diapause. It is not clear whether the determination of diapause in this generation is entirely endogenous, or whether only the reaction to environmental factors differs.

However, the possibility is not excluded that Central Europe represents a transitional area climatically (Bodenheimer & Vermes, 1957) between the regions settled with univoltine and polyvoltine populations of *C. septempunctata*. The hereditary tendency to diapause would have been gradually excluded in the first generations of the culture.

Of other insects undergoing imaginal diapause, *Leptinotarsa decemlineata* Say has also been reared continuously over several generations. A certain proportion of the females in *L. decemlineata* enter diapause in each generation, partly due to the density of beetles in the rearing cages (De Wilde & Stegwee, 1958). The proportion entering diapause decreased from approximately 30% when they were reared in groups of 30—50, to less than 10% when reared singly. Thus, diapause in *C. septempunctata* could also have resulted from similar sub-optimal rearing conditions.

Imaginal diapause in *C. septempunctata* synchronises its life history with the seasonal change in environmental conditions. This results in the coccinellid being active when the environment is most favourable to it.

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ZUSAMMENFASSUNG

DIE VERHINDERUNG UND KÜNSTLICHE INDUKTION DER DIAPAUSE BEI COCCINELLA SEPTEMPUNCTATA *L. (COL.: COCCINELLIDAE)*

Die Aufzucht des Marienkäfers *Coccinella septempunctata* L. unter optimalen Laborbedingungen (Langtag, erhöhte Temperatur, Futterüberfluss) bewirkte eine stufenweise Unterdrückung der Diapause. Demzufolge konnten dann diapausefreie Generationen in lückenloser Folge herangezogen werden. Wenn es aber zu irgendeiner Abweichung von den optimalen Bedingungen kommt (z.B. vom Futterüberfluss), entwickelt sich auch in den nachfolgenden Generationen ein gewisser Anteil inaktiver Käfer.

Nach einer mehr als einjährigen Zucht (etwa in der elften oder zwölften Generation) wurde eine erhöhte Mortalität beobachtet. Die Zuchten würden deshalb in jedem Frühjahr durch im Freien gesammelten Imagines erneuert.

Versuche mit künstlich aufgezogenen Marienkäfern der späteren Generationen zeigten, dass bei der Diapause-Auslösung die Tageslänge eine entscheidende Rolle spielt. Diesem Befund zufolge verhindern Langtagbedingungen die Auslösung der Diapause sogar bei relativ niedrigen Temperaturen. Durch Kurztagbedingungen wird die Diapause ausgelöst, aber der Anteil der diapausierenden Weibchen steht in direkter Abhängigkeit von der Temperatur, indem dieser bei niedriger Temperatur erhöht, bei höherer Temperatur herabgesetzt ist.

Auf Grund vergleichender Sektionen und insbesondere biochemischer Analysen kann man vermuten, dass die physiologischen Verhältnisse bei den künstlich diapausierenden Marienkäfern denen der natürlich überwinternden ähneln.

Es fällt auf, dass im Freien bei dem überwiegenden Teile der ersten Generation die Diapause unter allen Bedingungen ausgelöst wird, die sich experimentell als optimal diapausehemmend erwiesen haben. Analoge Resultate haben wir auch in unseren Labor-Versuchen bei den ersten Generationen erhalten. Es ist bisher nicht klar, ob dieser Unterschied durch variable Sensibilität zu den Aussenbedingungen oder eher durch innere genetische Unterschiedlichkeit verursacht ist. Bei der letztgenannten Möglichkeit könnte eine Erklärung darin gesucht werden, dass man für die zentraleuropäischen Populationen von *Coccinella septempunctata* L. eine heterogene Zusammensetzung annimmt, die zum überwiegenden Teil von Monovoltinen und nur zu einem kleineren von Polyvoltinen gebildet wird.

Es zeigt sich, dass man durch präzise experimentelle Zuchten monovoltiner Insekten des gemässigten Klimas unter eindeutig optimalen Bedingungen latente Voltinismus-Tendenzen — wie z.B. potentiellen Polyvoltinismus, obligatorischen Monovoltinismus (verschiedenartig fixiert), bzw. ein Gemisch beider Typen —, welche in der Natur durch die Uniformität des Monovoltinismus verdeckt sind, aufklären könnte.

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ÜBER DIE BEDEUTUNG DER POLLENNAHRUNG FÜR *TYPHLODROMUS (T.) PYRI* SCHEUTEN (= *TILIAE* OUD.) (ACARI, PHYTOSEIIDAE)

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Für die überwinterten Weibchen von *Typhlodromus pyri* ist der frische Blütenpollen in den Apfelknospen eine ausreichende Nahrung. Sie ermöglicht auch bei Fehlen von tierischem Futter den Aufbau einer Raubmilbenpopulation. Die F1-Generation dagegen kann sich nur nach karnivorer Ernährungsweise fortpflanzen. Der bereits abgefallene und auf den Blättern befindliche Pollen dient für die überwinterten Weibchen nur als Notnahrung, die sie am Leben erhält, sie reicht aber nicht für eine Eiablage aus.

In der Literatur finden sich Angaben, dass die Raubmilben aus der Familie der Phytoseiidae vor allen Dingen räuberisch leben, dass sie sich aber auch mit rein pflanzlicher Nahrung, wie Blattsubstanz, Pilzen und Blütenpollen nicht nur entwickeln, sondern auch fortpflanzen können (z.B. CHANT 1959). Die in Stuttgart-Hohenheim durchgeführten Untersuchungen über die Lebensweise der Raubmilben erbrachten den Beweis der Notwendigkeit des tierischen Futters zum Aufbau einer Population (DOSSE 1959). Über die Möglichkeiten der Raubmilben, sich für einen längeren Zeitraum mit Blattsäften am Leben zu erhalten, wurde bereits berichtet (DOSSE 1959). Aber *Typhlodromus* (*T.*) *pyri* Scheuten, *T.* (*A.*) *finlandicus* (Oud.), *T.* (*T.*) *tiliarum* Oud. und *T.* (*A.*) *zwoelferi* Dosse stellten bei solcher Ernährungsweise die Eiablage ein und magerten sichtlich ab. Diese Nahrungsquelle reichte also nicht aus, um eine Fortpflanzung zu gewährleisten.

Bei dem Versuch der Fütterung von *T. pyri* und *T. zwoelferi* mit den auf Apfel vorkommenden Pilzen *Fusicladium dendriticum, Penicillium-* und *Aspergillus*-species kamen wir zu dem Ergebnis, dass dieses gebotene Futter überhaupt nicht angenommen wurde (DOSSE 1959).

Die dritte pflanzliche Ernährungsmöglichkeit der Raubmilben auf Apfel ist der Blütenpollen. Unsere ersten Untersuchungen, ob eine Fortpflanzung und Entwicklung bei reiner Pollennahrung gegeben ist, bezogen sich auf die bereits von der Blüte getrennten und auf den Blättern befindlichen Pollenkörner, weil zu dieser Zeit der grösste Teil der Raubmilben aus den Winterverstecken erschienen ist und auf den Blättern lebt.

Zur Klärung dieser Frage bedienten wir uns der früher beschriebenen und öfter erwähnten Käfige (umgewandelte Huffakerzellen, DOSSE 1957). Der Pollen wurde entweder auf leicht angefeuchtetem Papier gereicht oder auf den Blättern direkt. Als Versuchstiere benutzten wir *T. pyri,* die bei uns von den Raubmilben die grösste Bedeutung hat.

In beiden Versuchsreihen konnte beobachtet werden, dass die Weibchen der

Raubmilben den Pollen annahmen und ihn mehr oder minder stark aussaugten. Wir fanden in den Käfigen sogar völlig leere Pollenkörner vor, von denen nur die äussere Umhüllung übriggeblieben war. Trotzdem Blütenstaub in riesigen Mengen angeboten wurde, stockten Eiablage und Entwicklung. Die Milben blieben zwar mit diesem Futter wochenlang am Leben, jedoch legte keins der angesetzten überwinterten 20 Weibchen auch nur ein einziges Ei ab, und dabei handelte es sich einwandfrei um befruchtete Tiere, deren Fertilität vor Beginn der Versuche geprüft worden war. Das Problem dieser Pollenernährung von Apfelblüten liess sich bis in den Juli hinein verfolgen. Der abgefallene und auf den Blättern sitzende Pollen reicht mit seinen Nährstoffen nicht aus, um den Weibchen eine Eiablage zu ermöglichen. Aber auch die Entwicklung geht mit diesem Futter nicht voran. Die mit Pollen als einziger Nahrung versehenen 20 Nymphen starben sämtlich nach kurzer Zeit ab, ohne ein weiteres Stadium zu erreichen.

Die gleichen Ergebnisse erzielten wir bei den fertigen Weibchen wie bei den Nymphen, wenn wir die Pollenkörner auf den Blättern beliessen und also beides gemeinsam reichten. Wie bereits ausgeführt, stellt die Blattnahrung allein kein vollwertiges Futter dar, dass eine Eiablage und Entwicklung von *T. pyri* gestatten könnte. Aber auch mit Pollen plus Blatt liess sich beides in unsern Versuchen nicht erreichen. Die Zahl der Versuchstiere betrug auch hier in jeder Serie 20, insgesamt also wurde die Prüfung der Pollennahrung mit 80 Exemplaren durchgeführt. In keinem einzigen Falle liess sich ein Anhaltspunkt dafür finden, dass der von den Blüten gelöste und auf den Blättern befindliche Pollen mehr als eine Notnahrung darstellt. Praktisch gesehen kann man diese Pollen- und Blattnahrung in ihrem Einfluss auf die Raubmilben gleichsetzen.

Anders sieht das Bild aus, wenn den Raubmilben der frische, noch in den Antheren befindliche Pollen zur Verfügung steht. Unter den Verhältnissen von Stuttgart-Hohenheim kommt nach unsern Befunden ein bestimmter Blütenzustand für den Aufbau einer Raubmilbenpopulation in Frage. Noch voll geschlossene Blüten, bei denen die Pollenschläuche ineinander verklebt und mit Haaren bedeckt sind, eignen sich nicht als Nahrungsquelle. In unsern Käfigen behinderten die Haare die Tiere beim Laufen und erschwerten die Fütterung. In solchen, aus dem Freiland eingetragenen Blütenknospen waren auch niemals Milben zu finden.

Erst wenn sich die Blüten lockern, war der mit dem Nektar behaftete Pollen zur Ernährung der Raubmilben geeignet. Gaben wir die abgeschnittenen Pollenschläuche in die Käfige hinein, konnten wir eine Eiablage und Entwicklung von *T. pyri* bewirken. Wir benötigten für ein Weibchen bei jeder Fütterung etwa den Pollen einer halben Blüte. Er musste spätestens alle 2 Tage erneuert werden, da er bei Trocknung, wie die oben genannten Versuche zeigten, nicht mehr den Nahrungsansprüchen der Raubmilben entsprach. Die Eizahl von *T. pyri* lag aber bei alleinigem Pollenfutter erheblich niedriger als bei tierischer Nahrung.

Bei reiner Pollennahrung erhielten wir nur bei 3 Tieren insgesamt über 15 Eier, die Höchstzahl betrug 22 bei einem Weibchen gegenüber 58 mit *Tetranychus urticae*-Futter bei gleicher Temperatur. Die geringste Eizahl lag bei 4, im Durchschnitt legte ein Weibchen 12 Eier ab. Die mittlere Legeleistung pro Tag errechneten wir auf 0,5 je Weibchen, im Gegensatz zu 1,3 nach Fütterung mit *T.*

TABELLE I

Entwicklungsdauer und Eiablage von T. (T.) pyri *bei Blütenpollen- und* Tetranychus urticae-*Futter.*

	Zahl der Versuchstiere	Durchschnittl. Gesamteizahl	Durchschnittl. tägliche Eizahl	Höchste Eizahl	Gesamtentwicklung (Tage)
Pollennahrung	15	12	0,5	22	11,5
T. urticae-Futter	30	33	1,3	58	7,2

urticae. Die Lebensdauer der mit Pollen ernährten Weibchen war sehr unterschiedlich, die Legezeit erheblich kürzer als bei tierischem Futter (Tabelle I).

Die Entwicklung vom Ei bis zur Imago verlangte durchschnittlich 10—13 Tage bei einer Dauertemperatur von ca 24° C, also wesentlich längere Zeit, als wenn die tierische Nahrungsquelle zur Verfügung stand.

Während bei dargebotener *T. urticae* die erste Eiablage der Raubmilben kurze Zeit nach der Kopulation einsetzte, erfolgte sie nach Fütterung mit Pollen erst nach 3—6 Tagen. Die Eier wurden in den Versuchskäfigen auf die Pollenschläuche direkt abgelegt. Da durch Aufplatzen der Antheren nach unsern Beobachtungen die Eier z.T. zerdrückt oder so eingeengt wurden, dass die Larven nicht ausschlüpfen konnten, überführten wir sie zwecks Weiterzucht mit einem feinen Pinsel getrennt in frische Käfige und gaben erst nach Erscheinen der Larven Pollenkörner hinzu. Aus 95 Eiern entwickelten sich 64 Tiere dieser F_1-Generation bei reiner Pollennahrung zu Imagines, von denen waren 21 Männchen. Die restlichen Raubmilben starben als Nymphen ab.

Es gelang uns, die Angehörigen dieser F_1-Generation mit Pollennahrung zur Kopulation zu bringen. Hier aber mussten wir feststellen, dass von den insgesamt 43 Weibchen nur zwei zur Eiablage schritten. Dies liess den Gedanken aufkommen, dass die Weibchen nicht befruchtet sein könnten, da bei tierischem Futter die Eiablage ja bereits 2—3 Tage nach der Kopula beginnt. Auf zwei Wegen prüften wir diesen Umstand nach. Erstens wurde wochenlang nach der Kopula, nachdem regelmässig frischer Pollen gegeben worden war, ein Teil der Weibchen für eine mikroskopische Untersuchung geopfert. Diese brachte den Beweis einer Befruchtung, denn die Spermathecae waren mit Spermatophoren angefüllt. Ein anderer Teil der Weibchen wurde mit *T. urticae* weiter gefüttert, und sofort kam jetzt die Eiablage in Gang. Dass die Weibchen der F_1-Generation also fortpflanzungsfähig waren, bestätigten beide Methoden. Die reine Pollennahrung reichte danach zur Aufzucht der Folgegeneration der Raubmilben nicht mehr aus, trotzdem uns durch die spätblühenden Apfelbäume reichlich Pollen zur Verfügung stand.

Nach diesen Laboratoriumsversuchen besteht für *T. pyri* also die Möglichkeit, in dem frischen Pollen eine Ersatznahrung zu finden, die die im Frühjahr aus ihren Winterverstecken erscheinenden Weibchen in Stand setzt, mit der Eiablage zu beginnen und eine Anfangspopulation ins Rollen kommen zu lassen.

Um diese Befunde für das Freiland zu überprüfen, haben wir von Beginn der Blütezeit im Jahre 1960 an laufend noch nicht voll geöffnete Blütenknospen auf einen eventuellen Besatz mit Raubmilben hin untersucht. In mehreren tausend

Blüten fanden wir neben Thripsen, *Triophtydeus* sp. und *Bryobia rubrioculus* (Scheuten)-Larven nicht mehr als 10 Raubmilbenexemplare, und diese nur in den aufblühenden. In einem einzigen Falle wurde ein Raubmilbenei in einer Blüte entdeckt. Da aber *Bryobia*-Larven nicht selten in oder an den Blüten sassen, lässt sich der Gedanke nicht ausschliessen, dass die Raubmilben dem tierischen Futter nachgegangen sein könnten.

In der Vegetationszeit 1960 begann die Blüte im Raum von Stuttgart-Hohenheim relativ früh und zog sich wegen des ungünstigen Wetters über einen langen Zeitraum hin. Also ist bei einem langsamen Blühverlauf für die überwinterten Raubmilbenweibchen genügend frischer Pollen vorhanden. Unter unsern Verhältnissen schlüpft aber *Br. rubrioculus* bereits vor der Blüte oder zum mindesten während dieser, und so ist auch eine tierische Nahrungsquelle stets gegeben, wenn die Raubmilben aus ihren Winterlagern erscheinen. Dies geschieht ungefähr zu der Zeit, wenn die Blütenknospen am Aufbrechen sind. Wo *Bryobia* aber fehlt und *Metatetranychus ulmi* Koch die Apfelbäume besiedelt, ist *T. pyri* wahrscheinlich je nach Witterung auf den Pollen angewiesen, da diese Schadmilbe und auch die Eriophyiden und Tarsonemen etwa zum gleichen Termin auf den Blättern auftreten wie ihre Gegenspieler.

Die die Winterverstecke verlassenden Raubmilben finden also nach unsern Erfahrungen auf den Apfelbäumen oft bereits geschlüpfte Schadmilbenlarven vor, sodass im Freiland die beiden Nahrungsfaktoren tierischer und pflanzlicher Herkunft nicht streng voneinander abzugrenzen sind. Und da der Pollen nur in einem bestimmten Entwicklungszustand zum Aufbau der Raubmilbenpopulation geeignet ist, hängt es ganz von der Frühjahrswitterung ab, inwieweit er unter natürlichen Bedingungen angenommen wird und wie gross seine Bedeutung für die Raubmilben ist. Dass es diesen gelingt, den Aufbau ihrer Population zu beginnen, auch wenn noch nicht genügend Larven aus den Wintereiern der Schadmilben geschlüpft sind, beweisen die Laboratoriumsversuche. Diese Tatsache wird auch durch die Freilandbeobachtungen bestätigt.

Chant (1959) ging bei seinen mit *T. pyri* in England durchgeführten Versuchen ebenfalls der Frage der pflanzlichen Ernährung nach. Bei ausschliesslicher Blattnahrung erzielte er keine Eiablage der Raubmilben, bei Fütterung mit Apfelpollen dagegen legten die Weibchen in geringem Umfange Eier ab. Gemeinsam mit Fleschner (1960) in Kalifornien vorgenommene Untersuchungen zeigten, dass andere *Typhlodromus*-Arten, *T. (A.) hibisci* Chant und *T. (A.) limonicus* Garman & McGregor mit Pollen von Orange- und Avocadoblüten 0,3 bzw. 0,7 Eier pro Tag abzulegen imstande waren. Diese Befunde decken sich mit den eigenen, an *T. pyri* gemachten Erfahrungen.

SUMMARY

THE IMPORTANCE OF POLLEN AS FOOD FOR THE CARNIVEROUS MITE TYPHLODROMUS (T.) PYRI *SCHEUTEN (=* TILIAE *OUD.) (ACARI, PHYTOSEIIDAE)*

Laboratory and field observations show that fresh pollen, attached to the anthers in apple flower buds, may supply an adequate diet for overwintering females of *Typhlodromus (T.) pyri* Scheuten (= *tiliae* Oud.). This diet supports egg-production and development, so

that a population of this predatory mite is built up in the absence of animal food. However, the F1 generation cannot reproduce unless it feeds carnivorously.

Pollen shed on to the leaves is eaten by overwintering females; they are kept alive by it, but do not lay eggs without further animal food.

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STUDIES ON THE BEHAVIOUR OF *OSCINELLA FRIT* L. (DIPTERA) ADULTS OF THE PANICLE GENERATION

BY

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Evidence from direct observations in the oatfield and from catches in various traps shows that *Oscinella frit* adults of the panicle generation, emerging in the oatfield, have a high level of migratory activity.

About thirty years ago there was a running controversy between KÖRTING (1931) and RIGGERT (1931, 1935) as to the powers of flight and the dispersal of the frit fly, *Oscinella frit* L. Körting believed that flight was localised, with small hops; Riggert that is was more extensive. The matter was not finally settled by these workers, but students of insect dispersal in general, HARDY & MILNE (1938) and FREEMAN (1945) recorded *O. frit* abundantly in tow nets up to 660 and 90 m (2,000 and 277 feet) respectively on the English eastern coast. Also GLICK (1939) has recorded undetermined species of *Oscinella* at great heights over Louisiana, U.S.A. More recently TAYLOR (1960) has explained the different results of Körting and Riggert in terms of weather and trap efficiency. The extent of dispersal in *O. frit* has however remained unknown and this problem was alluded to by IBBOTSON (1958) in his work on adult behaviour in relation to weather.

Early in our work on the numbers of *O. frit* in an oatfield we were confronted with the problem that although the total emergence from the oat panicle varied between 1000—4000 per square metre, it was never possible to discover more than about 300 adults per square metre on any day. As adults live for at least ten days (RIGGERT, 1935) it seemed unlikely that they died in the field; the alternative conclusion is that they migrated. A movement of such large numbers, if limited in extent, might be expected to lead to a gradient in subsquent larval infestation away from the field; we were unable to discover such a gradient and concluded (JEPSON & SOUTHWOOD, 1958) "that the flies probably disperse from the oatfield, but over a very wide area".

However, it is important, from both the agricultural and the academic angle, to determine if large numbers of *O. frit* regularly migrate away from the oatfield producing them and whether such movements are localised leading to the build up of excessive populations in nearby grasslands. Or alternatively whether such migration is by way of high altitudes and thus is even more extensive than thought by Riggert, but analogous to that of many other insects, especially aphids (GLICK, 1939; JOHNSON, 1954, 1959; TAYLOR, 1958), when the *O. frit* population over large areas must be considered as freely interchangeable.

In two papers, (the present one and JOHNSON, SOUTHWOOD & TAYLOR, in prep.), the extent of migration in the panicle generation has been shown, from direct and circumstantial evidence based on observations in and around the oat-field and from catches in suction traps in the upper air. During these studies some information has also been obtained on another controversial topic, the feeding of *O. frit* in the field, and on other aspects of the adult behaviour.

METHODS

The work was done at Imperial College Field Station, Silwood Park, Ascot, Berkshire. The oatfield, 5 acres in extent, was on the East acing side of a shallow valley and the variety Blenda was sown on 22 April, 1959.

Continuous direct observations were made in the field from 07.00 hours (G.M.T.) to 20.00 hours (dusk) on 23 July, from 04.00 to 07.00 hours on 24 July and from 04.00 to 19.30 or 20.00 hours on 28 and 30 July and 7 August. The observations were of two types:

a. continuous observations — the same area of oats was watched continuously for fifteen minutes and the behaviour of each individual fly recorded.

b. rapid observations — the flies on an area of oats were simply counted; this took just over a minute. Any take-offs or landings that occurred during the counting were recorded. Seven rapid observations were made in succession in different parts of the field.

In both cases the observer carefully approached part of the field and visually delimited an area of about a sixth of a square metre. One continuous observation, which gives a measure of individual activity, was made every hour (at about the half-hour). Two groups of rapid observations were made in each hour, before and after the continuous observation (i.e. a total of 14 in one hour). Such replication gives a more reliable estimate of the behaviour of the population as a whole than the continuous observations, but it does not show, for example, whether take-offs are confined to part of the population or whether such behaviour is universal. From dawn to dusk on 7 August only one group of seven rapid observations was made in each hourly period.

An estimate of the number of adults emerging from the panicles was obtained by cutting all the panicles from five separate 30 cm lengths of row. These were placed in a muslin bag which was suspended in light shade from the branch of a large oak tree. The emerging flies were counted every day at 09.00 hrs, when the old panicles were thrown away and replaced by new ones cut from the field. Five such muslin bags of panicles were collected daily from 3—6 August. The total emergence, for the season, was estimated by bagging panicles as 2214 flies per square metre, whilst a count of empty puparia in the panicles at the end of the season gave an estimate of 2167 flies. The method therefore seems reliable.

The actual population of adults present in the field in the morning was estimated by placing pyramidal tents, which covered 0.84 sq. metre (1 sq. yd) at dusk and counting the enclosed flies in the morning at about 10.00 hours. This method is based on that of MACLEOD & DONNELLY (1957) and will be described in more detail elsewhere (SOUTHWOOD & JEPSON, in prep.). More or less absolute estimates of the flying population 1.3 m above ground in the oatfield and at three locations in the vicinity were made with suction traps. The exact positions, type of trap and the air delivery (JOHNSON & TAYLOR, 1955; TAYLOR, 1955) are given in Table IV. They were emptied at 09.00 hrs each morning, except weekends, so the catch on any one day is really the catch from 09.00 hrs that day until 09.00 hrs the following day, and as *O. frit* has a morning peak of flight, some of this will be included in the previous day's catch. This is important when considering Fig. 2. The oats were about 0.8 m high and so the mouth of the trap was about 0.5 m above the top of the crop.

Relative estimates of the flying population at various distances from the field were obtained using water traps. These were metal baking tins 29 × 19 × 6.5 cm, painted white inside and black outside and held about 90 cm above the ground on an iron stake and half filled with water containing a little detergent. Three traps were placed at 4.5, 18 and 45.5 m from the margins of the oatfield on each of four transects running radially NW, NE, SW, and SE away from the corners of the field.

Wind direction and speed at 91 cm above ground level was measured at the edge of the crop by a recorder of the type described by LEWIS & SIDDORN (1960) and temperatures in a Stevenson screen.

EMERGENCE AND THE TENERAL PERIOD

Detailed observations were made at 20° C in the laboratory on separate days on three flies (2 females, 1 male) whose emergence times were known exactly. The rate of development was strikingly similar in all cases, so further replication was deemed unnecessary for the present purpose. Their appearance and behaviour at various times after emergence was as follows:

On emerging from glume: entirely bright yellow, only legs and eyes partly darkened; wings unexpanded; ptilinum expanded; terminal segments of abdomen of female fully extended.

15 min.: able to run actively.

30 min.: femur, tibia and ocellar triangle black, rest of head and dorsum of thorax grey-yellow, abdomen yellow; wings expanded, milky; much rubbing of abdomen and wings with hind legs and of head with front ones.

40 min.: terminal segments of abdomen of females retracted in about two movements to normal position.

1 hr.: wings still markedly milky.

1 hr. 30 min.: dorsum of abdomen brownish; ptilinum only about three-quarters original size.

2 hrs: wings still very slightly milky, buzzed for first time.

2 hrs 30 min.: occasional short hopping flights; ptilinum projecting only by length of third antennal segment.

3 hrs 20 min., to 4 hrs: flew vigorously away towards window; head, thorax and dorsum of abdomen completely dark.

The colour of the abdomen of *O. frit* gradually darkens becoming a deep orange in the mature fly: attempts to relate the colour to a precise chronological scale have so far been unsuccessful.

During the days of the observations, except 30 July, the temperature in the field rose from about 11° at sunrise to about 23° at 10.00 hours (Fig. 3); as the flies sit on the oat glumes and face towards the sun their own temperature might be a degree higher than that of the ambient air. Thus in the field on these days the teneral period is likely to last about 4 hours and it is only during the first hour and a half of their life that tenerals can easily be identified. The total number of such young tenerals seen in each half-hour period during the days of continuous observation is shown in Fig. 1. The first teneral was noted from 20—50 minutes after sunrise and the last one five hours after sunrise.

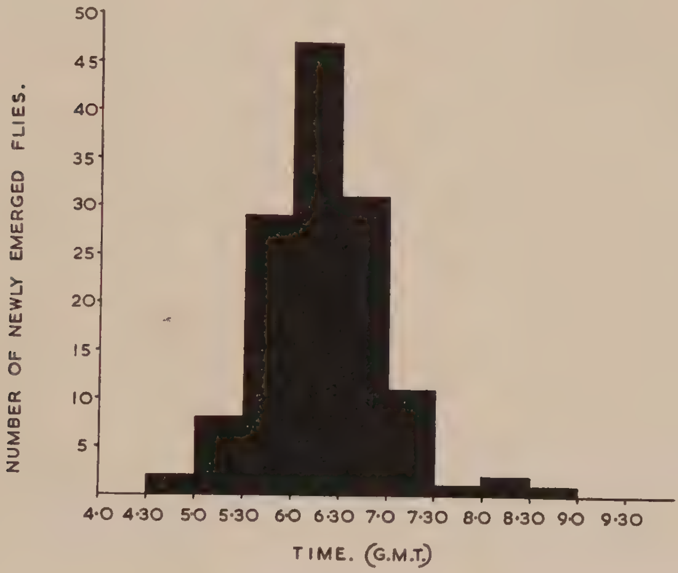

Fig. 1. The total number of newly emerged *O. frit* seen in each half-hour period during the days of dawn to dusk observation.

Confirmation that the great bulk of the emergence occurs in the early morning was obtained from the bagged panicles. From 27 July to 9 August the bags were emptied at about 21 hours as well as at 9 hours, and the total emergence in the period 09.00—21.00 hrs was but 28 flies, whilst 492 flies (many of them still teneral) emerged between 21.00 hrs and 09.00 hrs the next morning. This small emergence after 09.00 hrs was the "tail" of the morning emergence and not a subsidiary evening one, for none of the flies found in the bag at 21.00 hrs was teneral.

The daily totals of flies from the bagged panicles gave a picture of the seasonal course of the emergence in 1959 (Fig. 2). It will be seen that after rising quickly to a peak on 26 July the numbers fell off slowly until early September. In the normal course of events many of the late emerging flies would have still been puparia at harvest.

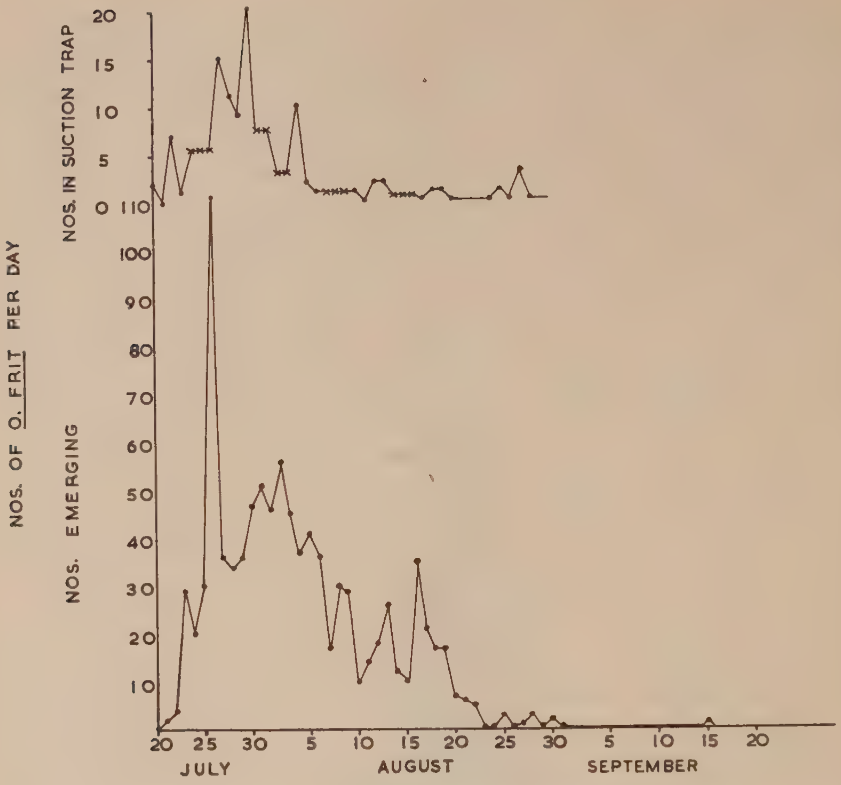

Fig. 2. Comparison of the number of *O. frit* adults flying and emerging in the oatfield. The numbers flying were estimated by trapping with a 9 in. Vent Axia suction trap and the numbers emerging by enclosing five separate 30 cm (1 ft) rows in a muslin bag. (x — mean values when catch for these days was collected together).

BEHAVIOUR IN THE OATFIELD

Numbers visible on the oats and frequency of take-offs and landings

Just before sunrise a few adult flies can be seen sitting on the outsides of the oat glumes, whilst further adults slowly ascend the oat stems or crawl out from inside the glumes. It seems therefore that some flies spend the night inside the glumes, others around the bases of the plants and yet others in an exposed situation. Around sunrise the flies are very lethargic and if touched will only hop. If undisturbed they soon crawl to the top of the glumes, or the stalk of the panicle and, if the air is still, face the sun.

About half an hour after sunrise the pale tenerals emerge from the glumes and take up a similar position. The first spontaneous flight occurs 1—1½ hours after the sun has just fallen on the panicles (i.e. 05.30—06.00 hours at the time of the present observations); this was also confirmed by the water trap catches (Fig. 4). The flies take off and fly upwards more or less towards the sun, generally they are soon lost to view; but occasionally in a momentary calm and

TABLE I

The take-offs and landings of O. frit *adults, as observed in the oatfield. (The rapid observation figures are based on data from 23, 24, 28 and 30 July and 7 August; those for continuous observations on data from 23, 24 and 30 July).*

	Rapid observations (approx. 1 min.)					Continuous observations (Two 15 min. obs. per hr.)		
Hour ending (G.M.T.)	Total no. of observations	Total flies observed	Mean no. flies/observation	Total no. of take-offs observed	Total no. of landings observed	Total flies observed	Total no. of take-offs observed	Total no. of landings observed
5	35	468	13.4	0	0	18	0	0
6	49	810	16.5	6	0	12	0	0
7	49	716	14.6	11	1	15	0	0
8	35	298	8.5	5	0	11	1	1
9	49	454	9.3	7	0	12	3	1
10	49	353	7.2	13	0	13	12	0
11	49	422	8.6	2	0	19	11	3
12	49	347	7.0	0	0	17	7	3
13	42	295	7.0	1	2	19	9	3
14	49	380	7.7	2	0	15	9	5
15	49	362	7.4	2	3	21	15	2
16	42	270	6.4	2	0	13	6	3
17	49	465	9.5	5	0	13	4	3
18	49	560	11.4	5	4	8	2	1
19	49	923	18.8	0	2	32	1	2
20	42	703	16.7	0	5	16	4	2
Totals observed		7826		61	17	254	84	29

with fortuitous lighting they can be seen flying in a wide spiral and disappearing from view at about 3 m above the ground.

The observed take-offs and landings of *O. frit* adults are given in Table I. A "take-off" was when the fly flew out of the area under observation, a landing when it arrived from outside and settled; short "hopping-flights" within the area were not recorded as take-offs or landings. It will be seen that the rapid observations show a majority of take-offs in the morning, with the main peak at 09.00—10.00 hours, and a majority of landings in the late afternoon. These trends are not so clear from the continuous observations. However, the values for the rapid observations are mostly based on 49 different samples and so are, *a priori,* less influenced by irregularities of population distribution, than the continuous observations which are based on but two samples. The fifteen take-offs noted at 15.00 hours in the continuous observations, for example, all occurred in one sample.

The rapid observations indicate a first wave of take-offs about two hours after sunrise, this could be due to those adult flies, a day or more old, that spent the night in the field. This early flight was not detected in the continuous observations and the first catch in the water traps outside the field (Fig. 4) was not until the period 07.00—08.00 hours. However, the population present on the oats does appear to fall sharply from 07.00 hours (as indicated by mean number of flies/

observation in Table I, i.e. the mean number visible/0.2 sq m during each hour).

A comparison of the number of take-offs with the mean number of flies/observation (Table I) indicates a high rate of take off. For example, at the peak, 09.00—10.00 hours, the total number of take-offs was 13 in a total observation time of 49 min., i.e. one fly took off about every 4 min. from a mean population of 7.2 flies (rapid observations) and 12 in 30 min. from a mean population of 6.5 flies (continuous observations). It therefore appears that every fly takes off at least once, the majority several times during the day; the population in any one spot presumably being replenished in the morning by the last flies to emerge and by the landings of flies from other areas. However, the same result could be obtained if the population consisted of a few very active flies and a large number of sedentary ones. That this is not the case and that a high level of flight activity is found throughout the whole population is confirmed by the continuous observations when the same individuals were watched for comparatively long periods.

It can be seen from Table I that take-offs and landings both occur more or less throughout the day, the take-offs reducing the number of flies visible on the oats (mean no. flies/observation) from about 07.00 hrs. The rate of take-off exceeds that of landing until about 17.00 hrs, when the mean number of flies/rapid observation starts to increase. The evening population, as indicated by mean no./observation, is as large or even larger than that visible at any moment in the morning. This is an artifact (see p. 204), and is probably due to many of the older flies taking off in the morning before a proportion of the young flies have emerged from the glumes.

During the hour or so before sunset the flies often buzz from panicle to panicle. They are last seen, after the sun has set, sitting quite motionless on the upper parts of the oat plant. The numbers are rather less than at an hour before sunset and presumably some have already gone into shelter. If touched they fly slowly in an arc settling again after a few feet.

The influence of weather on the number of flies on the oats

Three weather patterns occurred in the days of dawn to dusk observations. The 23 and 24 July and 7 August were mainly fine, sunny days, the ambient temperatures rising to 25° by mid-day. On these days the total number of flies visible on the crop was high in the morning and evening and low in the middle of the day when many flies were presumably flying (Fig. 3). On 7 August there was some light cloud until nearly 11.00 hrs and this appears to have slightly delayed the morning drop in numbers.

On 28 July the temperature rose to 23° by noon but there were several showers. The number of flies counted on the oats follows the U shaped curve noted on the other days (Fig. 3). But during the actual showers the numbers were slightly depressed and observations in the field showed that just as the rain started to fall many flies landed and made short, hopping flights down to the bottom of the plants, others ran down the stems and a few sheltered in the spikelets. Gusts of wind had a similar effect on their behaviour. When the sun came out again numbers of flies were very conspicuous running up the oat stems and sitting on the outside of the spikelets. This behaviour led, on 28 July, to a secondary peak at 14.00 to 15.00 hours.

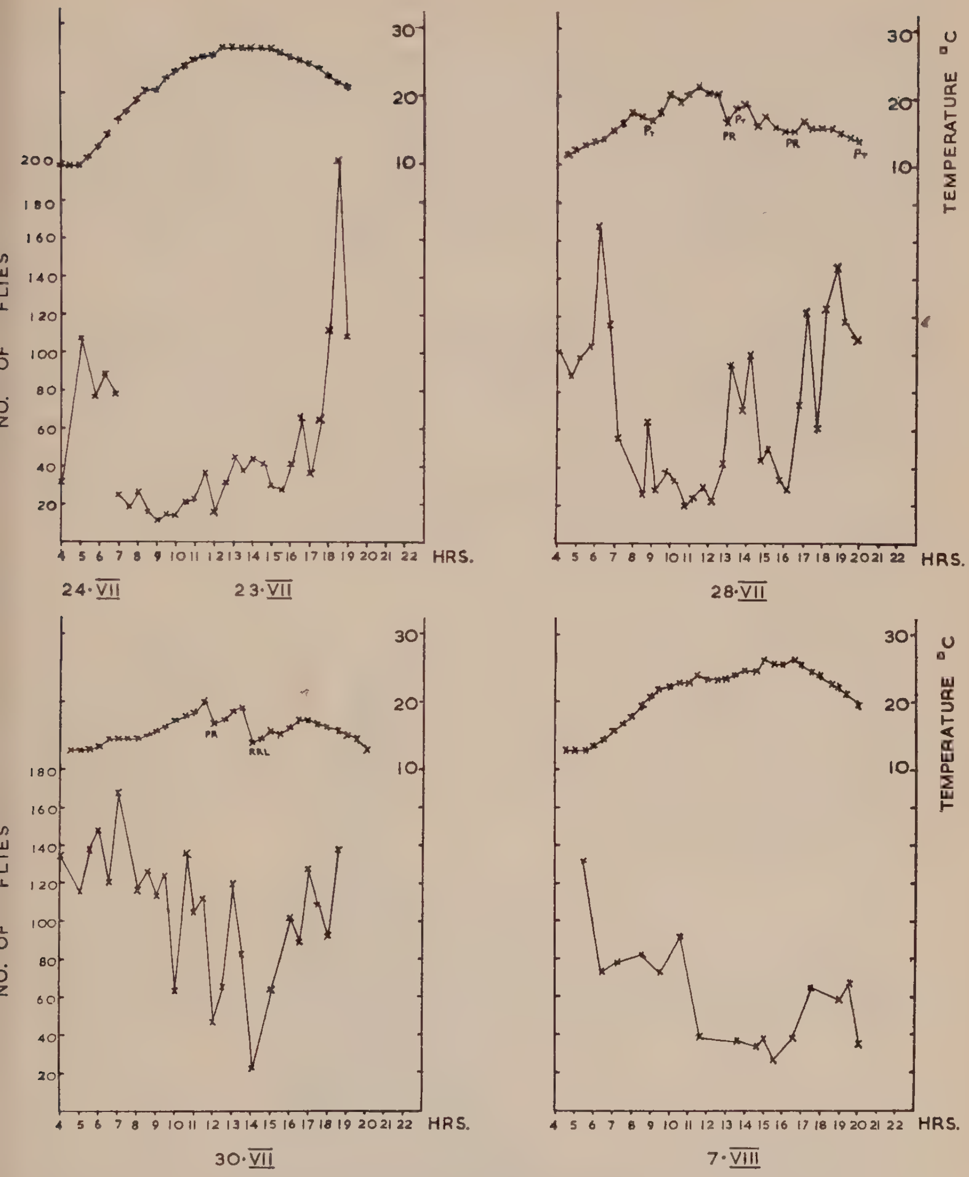

Fig. 3. The number of *O. frit* visible on the oats throughout four days, with the temperature graphs and times of rain (P, precipitation in form of: R, heavy rain; r, light rain; h, light hail).

The temperature on 30 July did not rise above 18° and it is noticeable that the U shaped curve for the total numbers was much more shallow than on the other days (Fig. 3). There were marked drops at 12.00 and 15.00 hours coinciding with showers; the number of flies visible rose quickly as soon as the shower was over.

These observations suggest that on a hot, fine day the population actually on the oats falls in the morning, presumably due to take-offs, and rises in the evening producing the U shaped curve. Showers produce a sudden drop in numbers visible, due to the flies hiding, and are followed by a rise in numbers sitting in exposed positions on the tops of the oats. These flies probably include some that were airborne before the showers. The U shape of the curve when all days are considered together can easily be seen from an inspection of the mean numbers of flies/rapid observation given in Table I.

Feeding

Flies were seen feeding at all times of the day from an hour after sunrise until sunset. In most cases they were feeding on minute dew or raindrops that had moistened a patch of aphid honey dew; they were also seen probing patches of mould and decaying plant tissue. Other workers (CUNLIFFE, 1921; RIGGERT, 1933) have generally considered flowers the source of food; however, there were very few flowers in or near the oatfield and in this instance aphid secretions were undoubtedly the main food supply, thus confirming the assumption of FINSLER (1924).

Copulation

Unlike feeding, copulation does not occur throughout the day; RIGGERT (1935) says from early mid-day to sunset. We observed it from 09.30 hours until sunset. Additional males may often be seen standing near a copulating pair. Nothing is known of the courtship of *O. frit* and so perhaps it is worth putting on record that once (at 09.15 hours) a male was observed sitting beside, but slightly behind a female and vibrating his head violently from time to time. Eventually the female walked away and then took off.

EFFECT OF MIGRATION ON POPULATION SIZE

An estimate of the total population in the oat field in the morning is obtained from the tents. This population consists of the overnight population, trapped when the tents were put out the previous evening, and the young individuals that have

TABLE II

The populations of O. frit *adults in an oatfield on three successive days, showing the morning to evening reduction due to migration (Number/sq. m with 5% fiducial limits)*

		4.VIII	5.VIII	6.VIII
I	Total morning population (observed — tents)	278 (184—366)	164 (102—210)	153 (98—200)
II	Total emerging (observed — bags)	113 (101—126)	81 (38—137)	104 (95—113)
III (I—II)	Overnight population (calculated)	165	83	49
Overnight population as % of previous morning population			29	36

emerged from their puparia early that morning. It has already been shown that the great majority of the daily emergence occurs before 10.00 hours when the tents were emptied. Now an estimate of the daily emergence is given by the bagged panicles and by subtracting this for any given day from the estimate of total population from tents, a figure is obtained for the overnight population, i.e. the population of adults in the field the previous evening. A comparison of this estimate with that for the total population the previous morning, from tents, gives a measure of the change in population size due to migration.

A series of such data for 4—6 August is given in Table II. It can be seen that the population in the evening is about one-third (36% and 29%) of that in the morning; the reduction is due to the balance between immigration and emigration. This is strikingly confirmed by the proportions of landings to take-offs in the direct observations (Table I), the landings being 31% of the take-off in the rapid observations, and 29% in the continuous observations. From the way that *O. frit* flies up into the air, from the catches in the upper air (JOHNSON, SOUTHWOOD & TAYLOR, in prep.) and from catches near the ground in other areas (SOUTHWOOD, unpublished) it seems likely that there will be quite a number of immigrants from other areas in the field at dusk. Therefore, at this season

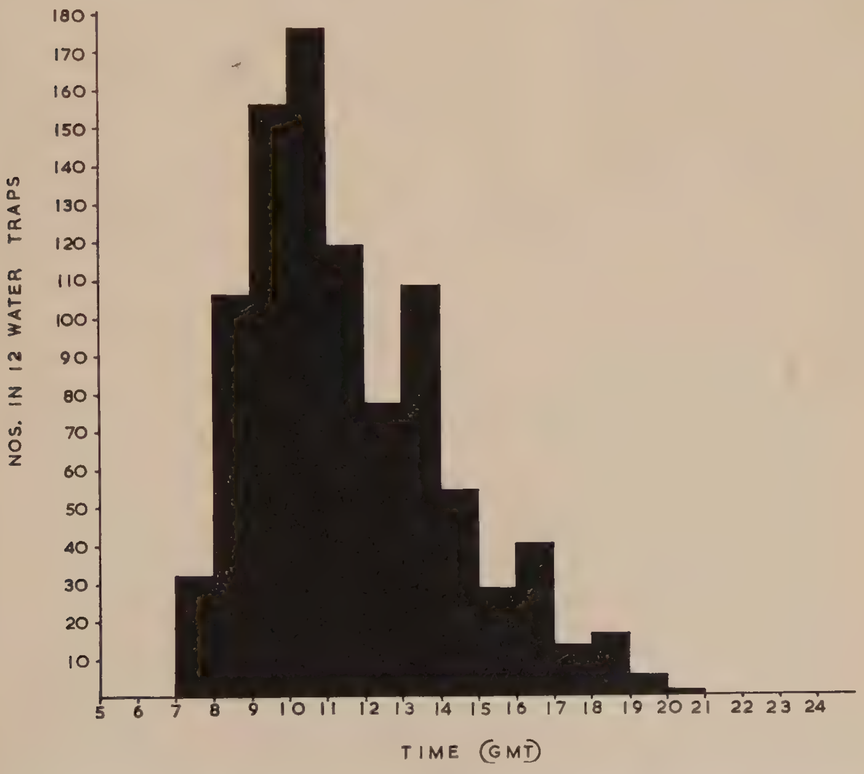

Fig. 4. The hourly totals of *O. frit* taken on 7 August in 12 water traps arranged radially round the oatfield.

and on a sunny day, of the flies present in an oatfield in the morning, at the most about a third, and probably fewer, of the same individuals will remain in the evening.

CATCHES IN SUCTION AND WATER TRAPS

Daily periodicity

On 7 August 1959, the 12 water traps arranged as described on p. 198 were emptied at hourly intervals. The results are shown in fig. 4 and indicate a peak between 08.30 and 11.30 hours. This agrees with the observation on the frequency of take-off (Table I) and reasonably well with the fall in numbers observed on the oats (Fig. 3).

The influence of wind direction on catches around the oatfield

On 28 and 29 July and 4—7 August, inclusive, complete day catches of *O. frit* were made in twelve water traps, arranged in threes radiating along transects running NW, NE, SE and SW from each corner of the oatfield (p. 198). An

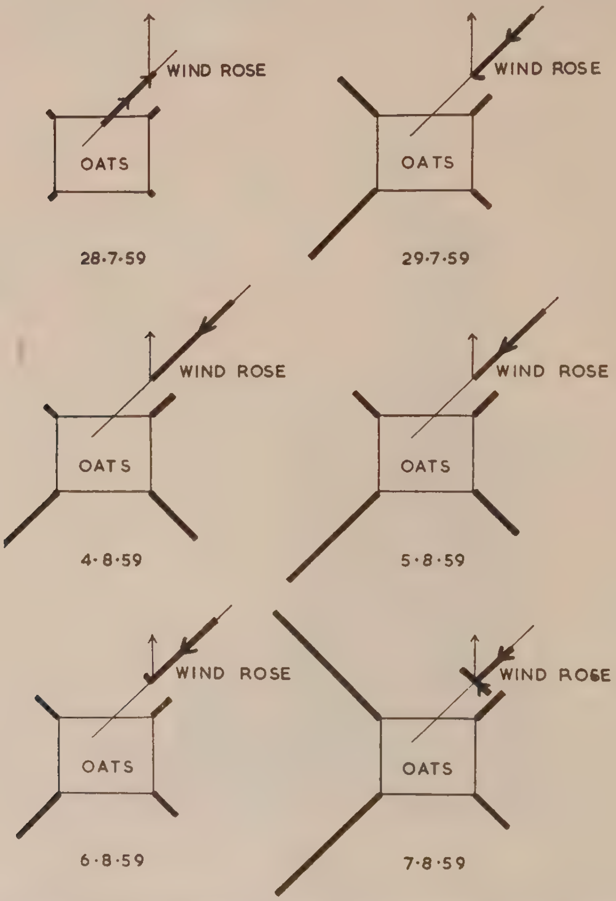

Fig. 5. The numbers of *O. frit* taken in water traps arranged radially round the oatfield and the wind roses for six days.

TABLE III

Analysis of variance of catches of Oscinella frit *adults in water traps arranged in transects running out from the oatfield*

	Degrees of freedom	Sum of Squares	Mean Square	Variance ratio	Probability
Distances	2	2470.8	1235.4	4.813	< 0.05
Direction	3	22421.0	7473.7	29.120	< 0.001
Dates	5	35962.7	7192.5	28.025	< 0.001
Distances × directions	6	10135.3	1689.2	6.582	< 0.001
Distances × dates	10	5791.1	579.1	2.256	0.05
Directions × dates	15	28697.7	1913.1	7.454	< 0.001
Residual	27	6929.6	256.7		

analysis of variance of the results (Table III) shows that direction and date account for most of the variability. The effect of distance was comparatively small, in fact the mean (50.5) of the traps at 18 m from the margin of the oatfield was higher, but not significantly so than that (45.6) of the traps at 4.5 m from the margin; the mean (36.4) for the traps at 45.5 was significantly lower, but still quite high.

If the wind roses for these days, based on the results from the recorder by the field, are compared with the total numbers of flies caught along each transect (Fig. 5) it is seen that the largest catches occurred along these transects where the wind was blowing from the field. The wind speed at 90 cm on all these days fluctuated between 1—3 km/hr. (1½—5 m.p.h.).

These results indicate that the oatfield was the source of the majority of the flies found in the water traps on these days, that the direction of migration is largely controlled by wind and that the aerial fly population does not fall off sharply with distance away from the oatfield.

Catches in suction traps in the oatfield and elsewhere

The catches of *O. frit* in four suction traps and the type and position of the traps are given in Table IV. Unfortunately the fact that the traps were not emptied daily makes an exact day to day comparison with emergence impossible. However, the trap in the oatfield does show a rapid rise in numbers between 23 and 27 July and a fall-off starting 5 August. If this is compared with the emergence curve (Fig. 2) a general similarity can be seen, indicating that the flies either cease to fly or leave the oatfield, within, at the most, a day or two after emergence. In contrast the traps outside the oatfield still caught flies in some numbers after 5 August; the trap quite close to the oatfield does show a sharp drop in numbers whilst the trap farthest away, amongst broom bushes, caught *O. frit* adults only after 6 August.

Thus these limited suction trap data support the view that the population of *O. frit* adults leaves the oatfield soon after emergence and may be found all over surrounding grasslands. A more detailed account of the flying populations of *O. frit* and other chloropids over grasslands and oats and their relationship to emergence will be published later.

TABLE IV

The catches of Oscinella frit *adults in various suction traps during the peak emergence of the panicle generation adults in 1959. (The traps were emptied at 08.00 hrs. G.M.T. and the date is that of the previous day. As the traps were not emptied daily the mean catches have to be worked out for certain days, these are marked *.)*

	Suction traps (position, type, air delivery)			
	Oatfield	Short grass 10 m due S. of oatfield	Long grass 170 m W. of oatfield	Broom bushes & long grass 190 m N.W. of oatfield
Date	9″ Vent axia 293 cu. ft./min.	18″ propeller type 2,510 cu. ft./min.	18″ propeller type 2,510 cu. ft./min.	9″ Vent axia 293 cu. ft./min.
23 July	1	1	6	0
24	5.7 *	7.7 *	4.7 *	0
25	5.7 *	7.7 *	4.7 *	0
26	5.7 *	7.7 *	4.7 *	0
27	15	4	6	0
28	11	4	14	0
29	9	1	9	0
30	20	7	28	0
31	7.5 *	31.5 *	24.5 *	0
1 August	7.5 *	31.5 *	24.5 *	0
2	3 *	29.5 *	19 *	0
3	3 *	29.5 *	19 *	0
4	10	76	25	0
5	2	56	7	0
6	1	18	25	0
7	1 *	20 *	21 *	5 *
8	1 *	20 *	21 *	5 *
9	1 *	20 *	21 *	5 *
10	1	9	7	0
11	0	10 *	14	5
12	2	10 *	26	0
13	2	10 *	9	2

DISCUSSION AND CONCLUSIONS

Observations in the field and the results from "bagged" panicles confirmed Riggert's statement that the great majority of *O. frit* adults emerge before 10.00 hours, the first tenerals being seen about half an hour after sunrise. Such newly emerged flies (i.e. up to 1½ hours old) were most abundant between 06.00 and 07.00 hours (Fig. 1). It appeared from direct observations in the field that every fly takes off at least once during the period 09.00—11.00 hours; there was a peak of take-offs during this period (Table I) and also a peak of catches in the water traps (Fig. 4). The population in the field in the morning is composed of a mixture of newly emerged flies and others at least a day old; it is tempting to link the peak of new tenerals (06.00—07.00 hours) to the peak of flight (09.00—11.00 hours) by the crudely determined teneral period of 3—4 hours. It seems, however, that both newly emerged and older flies must take off during this period. The exact relationship of developmental state and temperature to the periodicity of take-off requires further analysis.

The flies were seen to take off throughout the day; they mount to the top of the oat plant where they sit, generally facing into the sun or the wind; they then fly upwards into the air and, as their flight speed is about 2 m.p.h., at most times they will not be able to maintain controlled directional flight, but will be carried in air currents both horizontally and vertically.

In view of the observation that virtually all the flies take off in the morning, one might expect a considerable drop in total population. On two days it was found that the evening's population was but a third of that present in the oatfield in the morning; the number of flies seen landing in the field was also about a third of the number seen taking off. It cannot be claimed that even the reduced population present in the field in the evening is entirely composed of individuals that have remained in the oatfield all day.

The rise in the catches in suction traps around the field following the emergence of the panicle generation adults in the oatfield and the fact that the largest catches in the water traps around the oatfield were in those on its leeward side are further indications of extensive migration from the oatfield. The influence of wind direction on the water trap catches also supports the view that the flight of *O. frit* away from the field would not be controlled by the flies and the small effect of distance on the trap catches confirms JEPSON & SOUTHWOOD's (1958) supposition, based on samples of resultant larvae, that the migration from the oatfield is not just to the adjacent headlands but over a wide area. The parallel between the emergence curve (Fig. 2) and the numbers caught in the suction trap in the oatfield confirms the high level of migration.

The flies in the oatfield also engage in vegetative functions; feeding and copulation have often been observed and large numbers of eggs have been found laid on the occasional, green, anthesing oat panicles in a field ready for harvest. Whether these are autochthonous flies or immigrants is unfortunately unknown.

In conclusion, the present studies indicate that a high level of migration occurs in *O. frit* adults emerging from the panicles of oats. In hot, sunny weather this is expressed as a peak of take-offs in the morning, about 4½—6½ hours after sunrise and results in the evening population in the oatfield being much smaller than the total population present that morning. The very limited observations on the effect of weather on this phenomenon have shown that it is an important factor and in a cold, rainy autumn the detailed picture of the timing and possibly the extent of the migration would be considerably modified.

This work has been supported by grants from the Agricultural Research Council and the University of London Central Research Fund. It is a pleasure to express our gratitude to these authorities, to the head of our Department, Professor O. W. Richards, for his interest and support, to Mr. J. W. Siddorn for providing the meteorological data and to Dr. C. G. Johnson and Mr. L. R. Taylor for valuable criticism of the draft manuscript.

ZUSAMMENFASSUNG

UNTERSUCHUNGEN ÜBER DAS VERHALTEN VON OSCINELLA FRIT *L.-IMAGINES DER RISPENGENERATION*

Direkte Beobachtungen in einem erntereifen Haferfeld wiesen auf eine hohe Abflugintensität der Vollkerfe der Rispengeneration von *Oscinella frit* hin. In der untersuchten Periode lag dieser Höhepunkt morgens, ungefähr 4½—6½ Stunden nach Sonnenaufgang. Direkte Beobachtungen ergaben weiterhin, daß bei diesem Flug die Fliegen von Luftströmungen in horizontaler und senkrechter Richtung vom Feld weggetragen werden. Daß bei guten Wetterverhältnissen ein großer Anteil der Population abwandert, wurde durch aufeinanderfolgende Schätzungen der vorhandenen und der ausschlüpfenden Individuen im Haferfeld bestätigt sowie durch die rings um das Haferfeld aufgestellten, wassergefüllten Fallen. Es zeigte sich ferner, daß die große Mehrzahl von *Oscinella frit* am frühen Morgen aus den Puparien schlüpft und daß die Paarung nicht vor der Mitte des Vormittags stattfindet, womit die Angaben von RIGGERT (1935) bestätigt werden. Es wurde beobachtet, daß sich die Fliegen im Haferfeld von Honigtautropfen der Blattläuse und von verfaultem Pflanzengewebe ernähren. Sämtliche Stufen der Vorflugreifeperiode (teneral period), die bei 20° C 3—4 Stunden dauert, werden beschrieben.

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LOW TEMPERATURE THRESHOLDS FOR FLIGHT IN *APHIS FABAE* SCOP.

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The temperature thresholds for wing-beating and successful flight in alienicolae of *Aphis fabae* were found by flying tethered aphids in a falling temperature and by dropping free aphids in still air at different temperatures. The median temperature thresholds for wing-beating, horizontal and upward flight were 6.5°, 13° and 15° C respectively.

The temperature threshold for flight is apparently lower than that for take-off in *Aphis fabae* Scop., for alienicolae have been caught in flight traps at heights up to 600 m below 12° C (C. G. JOHNSON, unpublished data) although they do not take off from host-plants below 15.5° C (H. J. MÜLLER & UNGER, 1951; C. G. JOHNSON & TAYLOR, 1957). Aphids may often experience low temperatures during dispersal in the upper air, and the temperature threshold for flight, as distinct from that for take-off, may be an important factor affecting dispersal. The temperature thresholds for both wing-beating and effective flight were therefore studied.

The temperature threshold for a given activity differs in different individuals; thus in a population a temperature threshold takes the form of a frequency distribution (see C. G. JOHNSON & TAYLOR, 1957). Where temperature threshold is used here in reference to several insects, it is taken as the median of this distribution, i.e., the temperature above which 50% of the insects could beat their wings or fly.

MATERIALS AND METHODS

Alate alienicolae, reared at 20 ± 5° C on broad beans (*Vicia faba* L.), were kept after the final ecdysis on young bean plants in darkness for 1 day at 20° C; at this temperature they become flight mature within c. 14 hr. (TAYLOR, 1957). Some aphids were flown then, others were flown after being kept for a second day in darkness at 6 different temperatures between 5° and 30° C.

Tethered flight: To determine the temperature threshold for wing-beating, single aphids were flown suspended from pins (B. JOHNSON, 1958; COCKBAIN, 1961) in a wind-tunnel of 2.5 cm diameter glass tubing, enclosed in a glass jacket (Fig. 1). Air flow of 45—65 cm/sec. (corresponding approximately to free-flight speed at 20° C) was maintained by a compressor pump and regulated by a valve and flowmeter. The air was cooled by passage through tubular copper coils in a sodium chloride ice-bath, and the humidity raised by moist cotton wool in one section of the tube. By mixing warm (laboratory temperature) and cold air, constant (± 0.5° C) or falling temperatures (from 0.2—3.0° C/min) were obtained. Temperature and humidity were measured by wet- and dry-bulb thermistors 5 mm from the suspended insect. The apparatus was lit from above by a 200 watt mercury vapour lamp which gave a light intensity of c. 180 m-candles in the working section.

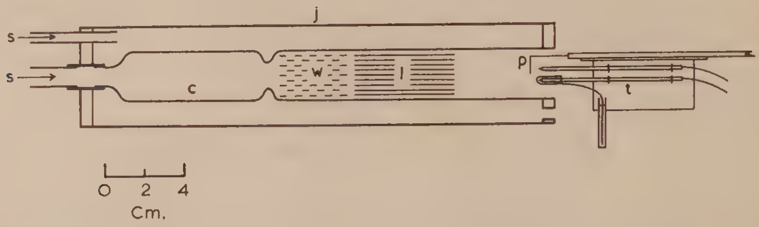

Fig. 1. The wind-tunnel; c, chamber for moist cotton wool; j, glass jacket; l, glass louvres; p, mounting pin; s, cold air-stream; t, wet- and dry-bulb thermistors; w, glass wool.

Free flight: To find the temperature threshold for forward and upward free flight, aphids kept at the temperature of the experiment for 10—20 min beforehand were dropped in still air of constant temperature; the number that flew downward, forward (± c. 10° from horizontal), or upward (± c. 80° from vertical) was recorded. Two 200 watt mercury vapour lamps were fixed above the release point and were insulated below by two perspex sheets, 3 mm thick, between which cold air was blown. Temperature and light intensity were measured at the dropping point (light intensity, 100—150 m-candles) and on the floor 1.6 m below (6—9 m-candles).

RESULTS

Temperature threshold for wing-beat

Tethered 1-day old aphids were flown in the wind-tunnel at 20° C; the temperature was then lowered at mean rates of 0.2—3.0° C/min. Some aphids stopped wing-beating when the temperature had fallen only a few degrees, but others "flew" continuously down to temperatures as low as 6° C; at the lower temperatures the wing-beat amplitude was noticeably smaller. Fig. 2 shows cessation of wing-beat at different temperatures; the mean air temperature at which this first period of continuous flight ceased was 11.6° C (range for 110 aphids, 5.3—18.4° C).

Some aphids resumed wing-beating spontaneously within a few seconds of stopping, but most required stimulation by touching their tarsi, or by moving them slightly in the wind-tunnel. The mean temperature at which further wing-beating could not be initiated in the wind-tunnel was 9.6° C (range, 5.0—18.4° C). Further flights were started, however, by removing the aphids, exposing them to warm air (c. 20° C) for a maximum of 10 secs, and then returning them to the air stream. The median temperature threshold for wing-beat (wing-beating maintained for 1 min) was 6.5° C (Fig. 2).

Two factors affecting the temperature threshold were the humidity of the air-

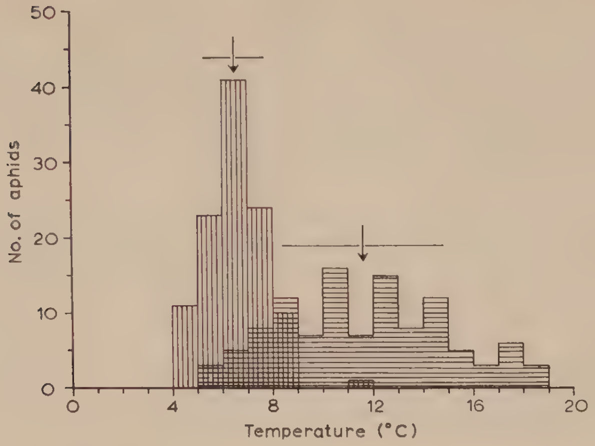

Fig. 2. Number of 1-day old aphids ceasing to beat their wings at different temperatures as the temperature dropped from 20° C. Areas with horizontal lines, temperatures at which the first period of continuous wing-beat ceased. Areas with vertical lines, temperatures below which wing-beat was maintained for less than 1 min. Mean values and standard deviations indicated by arrows and horizontal bars respectively.

stream and the rate of fall of the air temperature (Fig. 3). The regression lines for temperature threshold on rate of temperature fall differ significantly in position at the two humidity ranges but not in slope (Table I); the threshold was generally higher at low humidities (30 ± 3% R.H. at 20° C to 47 ± 4% R.H. at 10° C) than at high humidities (53 ± 6% R.H. at 20° C to 71 ± 7% R.H. at 10° C), possibly because of a greater lag between body and air temperature at the higher humidities.

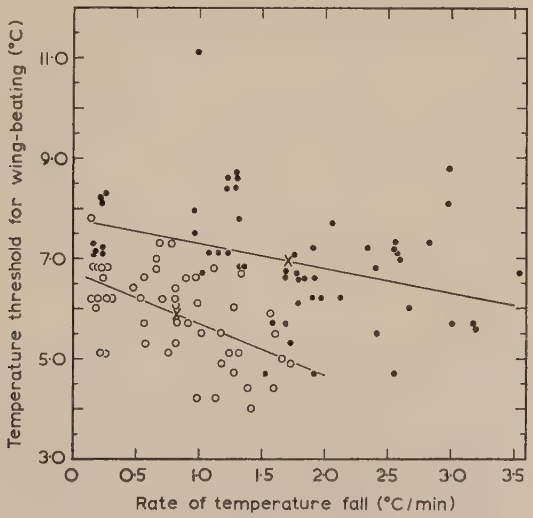

Fig. 3. Relationship between temperature threshold for wing-beat in 1-day old aphids and rate of temperature fall (from 20° C); ●, aphids flown at low humidities (r = —0.361; P = 0.01—0.001; b = —0.479); ○, aphids flown at high humidities (r = —0.543; P < 0.001; b = —1.040). For humidities see text.

The regressions also show that, at both humidity ranges, the temperature threshold became lower as the rate of cooling increased; fatigue was possibly a factor here, however, for aphids flown in a falling temperature of c. 0.2° C/min flew for more than 1 hr., to reach 7° C, whereas those flown in a falling temperature of 2.0° C/min flew less than 10 min.

TABLE I

Analysis of variance on the effect on the temperature threshold for wing-beat of rate of temperature fall at two ranges of humidity; tethered 1-day old A. fabae

	D.F.	Mean square	Level of significance
Single regression	1	0.531	> 0.20
Parallel regressions	1	50.387	< 0.001
Non-parallel regressions	1	2.821	0.10—0.05
Residual	106	0.894	

Duration of wing-beat movement at low temperatures

One-day old tethered aphids were stimulated to fly at room temperature and immediately placed in an air-stream of low constant temperature between 3° and 10° C. Table II gives means for the duration of the first "flight", and for the total flying time including first flight and all successive intermittent flights.

Different individuals behaved very differently at any one temperature, but, on the average, duration of wing-beating increased with increase in temperature. Continuous wing-beating was seldom maintained for more than 1—2 min below 7° C, and between 3—7° C most aphids flew with a very small stroke amplitude (10—30° in some, as against c. 160° at 20° C). Once the first period of flight ceased, further wing-beating could rarely be induced unless the aphids were exposed to warm air, but above c. 7° C intermittent flight was maintained for a time by frequent stimulation of the tarsi. Thus, the minimum and maximum durations at 9—10° C were 5 and 185 min respectively.

To compare these durations with those of aphids flown at higher temperatures, aphids reared in similar conditions were flown suspended from pins at 16—17° and 25—26° C in a flight chamber (COCKBAIN, 1961). The mean durations were 8.2 hr. (range for 12 aphids, 3.6—11.2 hr.) and 5.3 hr. (range for 27 aphids, 2.5—8.7 hr.) respectively. Tethered flight duration is probably limited at these temperatures by the amount of fuel available; below 10° C it is probably limited by the slowing down of neuro-muscular and metabolic processes below the level necessary to sustain wing movement.

Temperature threshold for forward and upward free flight

Two-day old aphids exposed to 10° or 20° C for the previous 24 hr. were dropped in still air at 9 different temperatures between 7° and 22° C. 97% started to beat their wings as they fell, but below 10° C none could check their fall. The mean rate of fall at 10° C was 75 cm/sec (range for 25 aphids, 45—112 cm/sec) compared with 208 cm/sec for anaesthetised aphids with their wings folded (range, 155—257 cm/sec.). Above 10° C, however, the number that checked their fall and then flew without losing height, i.e. horizontally or upward, increased sigmoidally with increase in temperature (Fig. 4A). The median temperature threshold for successful flight was 13° C.

TABLE II

Mean durations of wing-beat movement of 1-day old A. fabae *at low temperatures*

Temperature (° C)	3—4	4—5	5—6	6—7	7—8	8—9	9—10
No. of aphids	10	20	20	5	5	5	5
Mean duration and standard deviation of first period of continuous wing-beat (minutes)	0.3 ± 0.3	0.5 ± 0.7	2.3 ± 2.8	2.0 ± 2.4	4.4 ± 4.4	3.4 ± 1.7	14.8 ± 19.4
Mean sum and standard deviation of successive periods of wing-beat, including the first (minutes)	—	—	—	2.6 ± 2.1	10.2 ± 11.2	14.3 ± 16.3	63.7 ± 71.3

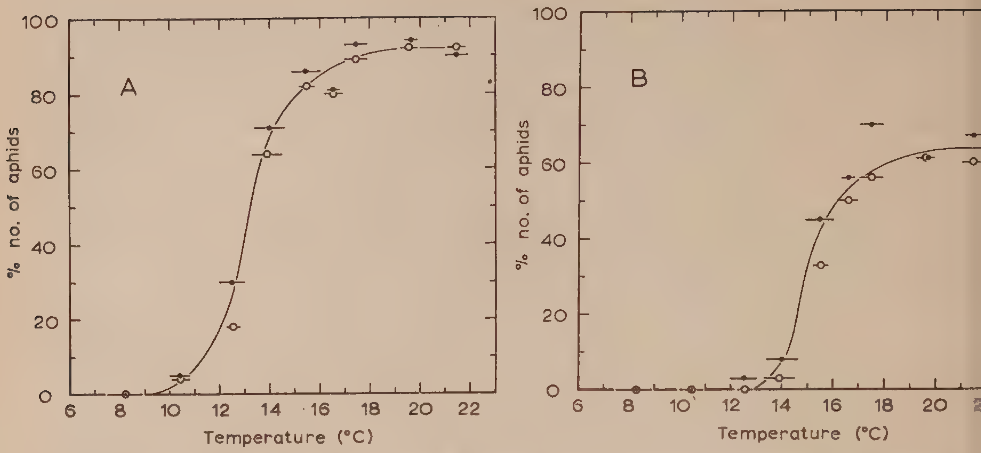

Fig. 4. Percentage of 2-day old aphids that flew (A) horizontally or upward, (B) upward, when dropped in still air at different temperatures; each point is based on 50—80 aphids; curves fitted visually. •, aphids exposed to 20° C for previous 24 hr.; ○ aphids exposed to 10° C. Temperature limits of experiments indicated by horizontal bars.

Not all aphids that fly and maintain height at a particular temperature can necessarily fly upward at that temperature, for the wing-beat movements may not produce sufficient lift. The sigmoid curve relating temperature to the number that flew upward (Fig. 4B) is at a higher temperature level than that for horizontal flight, upward flight apparently being inhibited in all aphids below 12° C, and in 50% below 16° C. Upward flight is a response to light, however, aphids being positively phototactic during early flight, responding particularly to blue-ultraviolet (KENNEDY & STROYAN, 1959); possibly in light of different intensity, or wavelength, the position and maximum height of the curve would be different. Thus, only 60% of the aphids tested at 100—150 m-candles and 19—20° C flew upward, but when aphids of the same age were tested in light above 300 m-candles at the same temperature, 81% flew upward. Assuming that all aphids fly upward at 19—20° C when the light attraction from above is sufficiently strong, the corrected median temperature threshold for upward flight is 15° C.

There was no evidence that aphids kept at 10° C for 24 hr. became acclimatized for flight at low temperatures (cf. MELLANBY, 1939). Generally more aphids from 20° C flew without losing height (Fig. 4A), or flew upward (Fig. 4B), at any one temperature, than those from 10° C; the difference, however, was not significant χ^2 for horizontal flight = 6.35, P = 0.70—0.50; χ^2 for upward flight = 9.08, P = 0.30—0.20).

To test further the effects of pre-exposure to different temperatures, 2-day old aphids kept for 24 hr. at four other temperatures between 5° and 30° C were dropped in still air at 12—13° C. The results (Table III) show no acclimatization by aphids kept at the lower temperatures, for the number that flew without losing height increased with increase in temperature up to 20° C; this was possibly because the metabolism of the aphids from the different temperature conditions had not become equally adjusted to the new temperature (12—13° C) when the aphids were tested. The percentage that flew successfully after exposure to 25°

and 30° C was significantly lower than after exposure to 20° C ($\chi^2 = 2.83$, $P = 0.10$—0.05 and $\chi^2 = 5.48$, $P = 0.02$—0.01 respectively), because most aphids kept at the two higher temperatures wandered off their plants during the second day in darkness and had starved for several hours, some for more than 20 hr., before flight.

TABLE III

Number of 2-day old A. fabae, *previously exposed to different temperatures for 24 hr., that flew horizontally or upward when dropped in still air at 12—13 ° C*

Temperature of previous exposure (° C)	5—6	10—11	14—15	20—21	24—25	29—31
No. of aphids	62	65	64	61	57	64
% with horizontal flight	11	18	20	26	14	13
% with upward flight	0	0	3	3	2	0

To test whether aphid age would affect the results, one experiment was done at 12—13° C with 58 one-day old aphids from 20° C; these had been flight mature for only c. 10 hr. when flown, whereas the 2-day old aphids had been mature for c. 1½ days (see TAYLOR, 1957). None flew upward, but 29% flew horizontally. This differs only slightly from results obtained with 2-day old aphids at this temperature, of which 3% flew upward and 30% flew horizontally.

DISCUSSION

The median temperature threshold for wing-beating in alate alienicolae of *Aphis fabae* is 6.5° C (wing-beating maintained for 1 min.), but most alatae cannot support themselves in still air below c. 13° C. Possibly, in nature, flight movements below 13° C lower the sinking speed of the aphid and assist in dispersal by air currents, but results indicate that such movements generally last for only a short time (< 15 min.) below 9° C, even with frequent stimulation.

The median temperature threshold for horizontal flight is 13° C (complete inhibition below 10° C) and for upward flight 15° C (complete inhibition below 12° C). The higher threshold for upward flight is probably associated with the greater lift and consequently higher rate of metabolism required for this activity (see WEIS-FOGH, 1956).

The results show that, like some other insects, e.g., the tsetse fly (MELLANBY, 1936) and the desert locust (WALOFF & RAINEY, 1951), aphids can fly at temperatures below those at which they normally take-off, the frequency distribution of spontaneous take-off against temperature in *A. fabae* having a mode at 17.3° C (complete inhibition below 15.5° C) (C. G. JOHNSON & TAYLOR, 1957). The lower temperature threshold for flight, e.g., upward flight, as compared with that for take-off probably reflects the greater flight-inducing stimulation (lack of tarsal contact, air movement, etc.) that an aphid receives when free in the air as against when it is on a host plant. The difference may also be associated with the internal warming-up of the insect by the metabolic heat produced during flight, but the increase in thoracic temperature of a small insect by this process can be only slight (see SOTAVALTA, 1954).

The temperature thresholds for horizontal and upward flight are based on the first few seconds of flight, and may not be applicable after prolonged flight. Thus the lift produced during early flight in *A. fabae* is relatively high, decreasing as flight continues (KENNEDY, 1958). If the lift during the first few seconds is only just sufficient to support the aphid in the air, then with decrease in lift the insect would lose height; effective flight may then require a higher temperature. Other factors may also affect the thresholds, especially those which cause differences between body and air temperature, e.g., radiation and evaporation. It is doubtful, however, whether either of these processes cause temperature differences of more than 1—2° C (see DIGBY, 1955; CHURCH, 1960) in a flying insect as small as *A. fabae.* Their effect on the thresholds would probably not be more than this.

The ability to fly successfully at low temperatures is affected by the temperature experienced before flight, though not in the direction expected from acclimatization (see Table III); this result may be peculiar to the present experiments, however, and is probably irrelevant in nature. There may be no effect if aphids transferred from low temperatures, e.g., 5° C, are allowed a longer time ($>$ 10—20 min) for their metabolism to become adjusted before flight, or if those from high temperatures, e.g., 30° C, are not starved. It is probable that in nature the temperature threshold for effective flight is unaffected by previous temperature (see MELLANBY, 1939).

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ZUSAMMENFASSUNG

UNTERE TEMPERATURSCHWELLEN FÜR DEN FLUG VON APHIS FABAE *SCOP.*

Die Temperaturschwellen für Flügelschlag und erfolgreichen Flug wurden bei alienicolen *Aphis fabae* Scop. an gefesselten Aphiden bei fallenden Temperaturen ermittelt sowie durch Fallenlassen freier Blattläuse in ruhige Luft bei verschiedenen Temperaturen.

Die mittlere Temperaturschwelle für Flügelschlag betrug 6,5° C (Flügelschläge eine Minute andauernd) und wurde vom Feuchtigkeitsgehalt der Luft sowie von der Abkühlungsrate (sowie möglicherweise von der Flugdauer) beeinflußt. Unter 9—10° C hielt der Flügelschlag nur für eine kurze Zeit an und die Schlagamplitude war bei diesen Temperaturen im allgemeinen gering.

Die mittlere Temperaturschwelle für horizontalen Flug lag bei 13° C (unter 10° vollständige Verhinderung) und für Aufwärtsflug bei 15° C (unter 12° völlige Hemmung); die höhere Schwelle für den Aufwärtsflug steht wahrscheinlich mit höherem Stoffwechselbedarf in Verbindung. Nach einem Aufenthalt von 24 Stunden bei niederen Temperaturen (5—15° C) vor dem Flug flogen bei 12—13° C weniger Blattläuse, als wenn sie bei 20° C gehalten worden waren. Diese Ergebnisse gelten nur für die ersten wenigen Sekunden des Fluges.

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THE OCCURRENCE OF A PARTIAL FOURTH GENERATION OF *OSCINELLA FRIT* L. (DIPTERA: CHLOROPIDAE) IN SOUTHERN ENGLAND

BY

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The occurrence of an additional generation in oats of the frit fly (*Oscinella frit* L.) is reported. The partial emergence of the generation and the high rate of larval mortality are discussed.

The number of generations of the frit fly (*Oscinella frit* L.) in a year appears to vary with temperature. There are normally two in northern England (IBBOTSON, personal communication) and in Russia one in the north but as many as four in the south (KURDJUMOV, 1913).

In the south of England the fly normally completes three generations in a year; the two summer (tiller and panicle) generations may be passed in spring oats or in grasses, but the third (overwintering) generation is normally confined to grasses because of the absence of oats after harvest. Here factors restricting the population build-up of the fly appear to operate (SOUTHWOOD & JEPSON, 1961).

The summer of 1959 was exceptionally hot and at the Imperial College Field Station, Silwood Park, Sunninghill, Berks. the adults of the panicle generation emerged in mid-July, two or three weeks earlier than normal. Spring oats were sown in an area adjacent to the main experimental oatfield during this month in an attempt to confirm that *O. frit* would attack oats at this season and to determine whether the population checks which appear to act in the grasslands during the autumn would also occur in oats.

In fact these late oats were attacked by *O. frit* and a partial fourth generation resulted. The occurrence of this extra generation is considered worth reporting, especially as several features of general relevance to work on *O. frit* were demonstrated.

MATERIALS AND METHODS

The oats were sown broadcast on an area of 80 sq. metres on the north side of the main experimental oatfield. The poor stand of approximately 120 stems per square metre was heavily attacked by *O. frit.* About 75% of the plants were affected, and plants containing up to seven larvae were found in early August.

12 samples, each consisting of 30 oat plants, were taken at intervals from August 1959 to January 1960. The plants were selected by pacing along a zig-zag path covering the whole plot, and uprooting the plant nearest to the leading foot

every third pace. The plants were dissected in the laboratory, and the numbers of deadhearts, larvae, pupae and empty pupae were recorded. All pupae were dissected for parasites, and the apparently healthy pupae were classified according to the amount of development on the following scale:

1. Pupa not yet formed within the puparium.
2. Pupae formed, but eyes of pharate adult not pigmented.
3. Eyes of pharate adult pigmented, but little additional pigmentation on body.
4. Body of pharate adult almost entirely pigmented.

To check the order of magnitude of the numbers of empty pupae, two samples of 30 plants collected weekly were kept in preserving jars with trap lids to catch emerging *O. frit*. The number of larvae at the end of October was checked by placing ten samples of 30 plants in biscuit tins. Emerging adults were caught in glass traps mounted over holes in the lids of the tins. The tins were kept under a 250 watt bulb in a Constant Temperature room at 25°.

The distribution of both larvae and pupae in oat stems was considered as effectively random, as the variance of 12 samples of 30 plants was close to the value of the mean. The authors feel that since the arithmetic mean of the sample counts affords an unbiased estimate of the density of the insect per unit area, such means should be adhered to. Fiducial limits ($p = 0.05$) for a Poisson series can be calculated (PEARSON & HARTLEY, 1958; p. 75) and samples of such limits are given (Table I) together with the detransformed square root mean more familiar in this context and its limits.

TABLE I

Sampling error of means of 12 samples of 30 plants.

Item	Period	Arithmetic Mean	Fiducial limits (Poisson series)	Detrans. sq. root mean	Detrans. sq. root fiducial limits
Larvae	15/9— 2/10	4.83	(6.22—3.65)	4.65	(5.95—4.00)
Pupae	15/9— 2/10	3.08	(4.25—2.15)	2.78	(4.25—1.57)
Larvae	28—30/10	7.33	(9.01—5.86)	7.12	(8.80—5.60)
Flies emerged from tins	28—30/10	7.70	(9.64—6.05)	6.84	(10.96—3.58)

The number of plants per square metre was assessed at intervals by counting plants within 100 1 sq. ft. (9.29 sq. decimetre) quadrats. The coefficient of variation ($p = 0.05$) of means based on 100 quadrats was calculated as 16.4%.

RESULTS

Detailed work was not commenced until late August, by which time there had already been a considerable decrease in the population of *O. frit* probably caused by a high death rate of oat tillers.

The data, expressed as means per 12 samples of 30 plants corrected to numbers/sq. metre are given in Table II.

Each pupa was given a score of 1—4 on the developmental categories listed above, and mean pupal development is expressed on this scale in the table.

TABLE II

Progress of 4th Generation O. frit. *Autumn and Winter 1959—1960 (Expressed as Nos./sq. metre).*

Period	25—28/8/59	28/8—8/9/59	15/9—2/10/59	28—30/10/59	20/1/60
Emerged adults		1.7 *O. frit*	3.5 *O. frit*		
Pupae	6.5	1.1 empty 1.3 parasitised 8.6 healthy 9.9 unemerged	5.3 empty 1.9 parasitised 3.3 healthy 5.2 unemerged	0	0
Mean pupal development (see p. 221)		1.7	2.4—2.9		
Larvae	6.3	0.5 parasitised 3.9 healthy 4.4	8.3 healthy	9.8 healthy 10.3 flies bred in tins	0.1 parasitised 0.2 with bacterial infection 1.9 healthy 2.2

The points of interest are as follows:

1) *The emergence of a partial fourth generation (Table II).* These adults emerged from 26th August to 22nd September.
2) *Development and temperature.* The continued development of pupae well into October confirmed that emergence was not limited by diapause up to that point. At the same time the temperature sum (based on a threshold of 7.2°) far exceeded figures established for the summer generations (JEPSON & SOUTHWOOD, 1958). It is thus somewhat surprising that the fourth generation was only partial, and not complete (Table III). This suggests that a new concept of effective temperature with emphasis on the non-linearity of the temperature/development rate curve may be necessary for a comprehensive forecast of emergence.

TABLE III

Temperature sums (threshold 7.2°) for tiller, panicle and fourth generation of O. frit

Generation		Day degrees
Tiller	(After JEPSON & SOUTHWOOD, 1958 modified for ° C.)	322
Panicle		356
1959—Fourth		
(to 8/9)		401
(to 15/9)		468
(to 28/10)		723

3) *Mortality.* Compared with the summer generations in the oatfield, mortality was approaching the level which appears to prevail in wild grasses over winter (JEPSON & SOUTHWOOD, 1958). The total mortality of *O. frit* from 25th August 1959 to 20th January 1960 was calculated as 45.7% (Table IV).

TABLE IV

Estimate of O. frit *mortality during 4th generation*

No. of individuals/sq. metre from 25th August, 1959 to 20th January, 1960.

	No.	%
Parasitised	2.0	9.4
Infected with bacteria	0.2	1.1
Disappeared (death of stems ?)	7.5	35.2
		45.7
Emerged as *O. frit*	9.7	45.4
Surviving as apparently healthy larvae	1.9	8.9

The death of plants during autumn and winter (Table V) appears to have been a major mortality factor. It seems plausible to suggest that plants attacked early by *O. frit* would be the first to succumb, especially as the number of deadhearts fell significantly faster than the number of plants ($p = < 0.001$). Measurable parasitism of larvae and pupae was observed in this generation. Although attack by parasites directly destroyed less than 10% of *O. frit,* the figure of 17.4% representing parasitisation of individuals escaping mortality as larvae is probably a more reliable estimate of parasite attack. Previous estimates of parasitism (BHATTACHARYA, 1956) have been based on spot checks of pupae near the end of a generation; such an estimate at the end of September 1959 would have given the high figure of 36.4%. 10.5% of the larvae remaining in January were infected with bacteria.

4) *Numerical discrepancies.* In the progress of the generation as recorded in Table II these fall well within the sampling error with the exception of variations in the numbers of larvae. The decline in numbers of larvae between October and January may possibly be ascribed to the death of stems shown in Table V.

TABLE V

Changes in Numbers of Oat Plants and Deadhearts/sq. metre August, 1959—January, 1960

Date	No. plants/ sq. metre	No. deadhearts
10th August, 1959	Circa 120	?
31st August, 1959	51.1	85.9
17th December, 1959	40.3	17.9
26th January, 1960	24.5	3.1

This decline in deadhearts would reduce a larval population of 9.8/sq. metre at the end of October to 1.7 in January, which compares favourably with an observed figure of 2.3. Similarly sampling error could not account for the apparent increase in numbers of larvae during September. It is suggested that there may have been an immigration of larvae from stems of oats and weed grasses dying with the decline of summer conditions. In early August, before the sampling routine was commenced, up to 7 larvae were found in single plants in the heavily infested stand of approximately 120 stems/sq. metre. These numbers had been drastically reduced by 31st August, and some of the larvae from dead stems may have successfully re-invaded oats at a later date.

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ZUSAMMENFASSUNG

DAS AUFTRETEN EINER PARTIELLEN VIERTEN GENERATION VON OSCINELLA FRIT *L. (DIPTERA: CHLOROPIDAE) IM SÜDLICHEN ENGLAND*

Die hohen Temperaturen des Sommers 1959, die ein frühes Schlüpfen der Vollkerfe der Haferrispengeneration der Fritfliege (*Oscinella frit* L.) bewirkten, ermöglichten auch die Entwicklung einer partiellen vierten Generation der Fliege in Hafer, welcher neben dem Haupthaferfeld im Juli ausgesät war.

Das Studium dieser zusätzlichen Generation erbrachte folgende Ergebnisse:

a) Die bisherigen Angaben über die zur Entwicklung von *O. frit* notwendigen Temperatursummen scheinen nur für Generationen bei normalem Anbau gültig zu sein.

b) Die Mortalität der Larven ist im Herbst und Winter selbst im Hafer viel größer als in einem Haferfeld während des Sommers. Sie war bedingt durch das Absterben der Halme und durch einen höheren Parasitierungsgrad, als er im Sommer im Haferfeld normalerweise auftritt.

c) Die Ergebnisse deuten darauf hin, daß sich mehrere Larven aus abgestorbenen Stengeln in noch lebende wieder eingebohrt hatten.

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WINGED APHIDS TRAPPED IN POTATO FIELDS, 1942–1959

BY

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Sticky aphid-traps were operated over potato crops at Rothamsted during 18 years, and elsewhere for shorter periods, to get information about the activity of *Myzus persicae* in relation to the spread of virus diseases. Numbers of total aphids, *M. persicae, Aphis fabae* and *Brevicoryne brassicae* caught per week are given. Annual rhythms in aphid numbers were unpredictable, although there was a tendency for *A. fabae* to be few during the year after one in which they had been numerous. *M. persicae* were few in summer when they and their predators or parasites had been numerous in spring, and were often numerous in summer when they had been few in spring. *A. fabae* were more numerous during most years than *M. persicae,* and *B. brassicae* usually scarce. Severe winters affected *M. persicae* more than the other two species.

In studying the epidemiology of diseases caused by aphid-transmitted viruses use has often been made of traps to find when aphids were flying. Several types have been used, of which the suction trap (JOHNSON, 1950) is undoubtedly the best. However, the spread of viruses in potato and sugar beet crops has been significantly correlated with catches of aphids on sticky traps (BROADBENT, 1950; WATSON & HEALY, 1953; HOLLINGS, 1957), so these have been retained throughout the work on potato virus diseases. Sticky traps were operated for different periods at many sites in England and Wales, including Rothamsted, during each of the 18 years of the work.

METHODS

The first sticky traps, devised by DONCASTER & GREGORY (1948), were cylinders 90 cm long and 13 cm in diameter, painted white, and coated directly with a grease-banding preparation. From 1946 onwards they were shortened to 30 cm and the grease was carried on a transparent plastic cover (BROADBENT, DONCASTER, HULL & WATSON, 1948). BROADBENT (1948a) concluded that a single trap, properly sited, was enough to show the main periods of aphid flight; he found that yellow-painted traps caught more aphids than white ones, so traps from 1948 onwards were painted yellow.

Traps were sited within potato crops with the base about 1 m from the ground; covers were usually changed at weekly intervals while potato plants were above ground and alive, and the total aphids counted. The aphids were cleared from the grease as described by BROADBENT *et al.* (1948). *Myzus persicae* (Sulzer),

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Aphis fabae Scopoli (group) and *Brevicoryne brassicae* (L.) were the only aphids identified to species throughout the work. *M. persicae* is the principal vector of viruses infecting potato, sugar beet and many other crops; *A. fabae* and *B. brassicae* are major pests of field crops in Britain; *B. brassicae* is a vector of viruses in brassicas, and *A. fabae* of viruses in sugar beet and legumes.

The acreage devoted to crops that act as hosts for these three species varied from year to year on Rothamsted farm, but *M. persicae* and *A. fabae,* which are polyphagous, had about 45 and 30 acres (18 and 12 hectares) of suitable host crops respectively; *B. brassicae* which is oligophagous, had only about 10 acres (4 hectares) of kale and other brassicas. *A. fabae,* but not *B. brassicae,* sometimes colonizes potatoes.

The catches for the years 1942—45 are from the records of Mr. J. P. Doncaster, most of which were quoted by DONCASTER & GREGORY (1948) and BROADBENT & DONCASTER (1949).

RESULTS

Tables I, II & III show the numbers of *M. persicae, A. fabae* and *B. brassicae* caught per week during each of the eighteen years at Rothamsted, and Table IV the total aphids caught per month. Catches on white traps during 1942—47 were multiplied by 1.5 to make them roughly equivalent to those caught later on yellow traps (BROADBENT, 1948a), and those during 1942—45 were divided by three to make them equivalent to catches on the shorter traps.

The most important factors controlling the size of aphid populations are the condition of the host plants, the weather, and the prevalence of predators and parasites. It is extremely difficult to assess the relative importance of these factors, or to measure their effect at any one time. When winged aphids develop and when they fly depend largely on the stage of growth of the food plants. We assumed that host plants were approximately similar in the same area each year at similar times, and made no attempt to disentangle the effects of weather directly on the aphids and indirectly through the plants.

Weather affects the development of all aphid populations, but all species will not be similarly affected by any one factor: for example, species that overwinter as eggs will be much less affected by a severe winter than those that overwinter mainly in the active viviparous form. A general picture of the weather changes in each year was obtained by plotting the differences from the long-term means of the weekly temperature and rainfall.

The predators and parasites trapped each week were not counted, but some indication of their relative abundance was obtained during counts of aphids on potato leaves made weekly to estimate the aphid population (BROADBENT, 1948b).

Our interpretation of the influence of the different factors on the numbers of aphids trapped year by year is set out below.

SEASONAL DIFFERENCES AT ROTHAMSTED

1942. The winter of 1941—42 was very severe and no *M. persicae* was found surviving outside. Summer temperatures and rainfall were about average. *M. persi-*

TABLE I

Weekly catches of M. persicae *at Rothamsted 1942—1959*

Week	1942*	1943*	1944*	1945*	1946†	1947†	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959
May 1	0	1	0	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—
,, 2	0	5	2	0	0	—	—	—	0	—	—	—	—	—	—	—	0	—
,, 3	1	1	2	0	2	0	0	9	3	—	—	1	—	—	0	—	0	1
,, 4	1	2	1	1	0	0	0	2	3	0	1	2	—	0	0	—	1	0
June 1	0	0	0	0	0	0	0	6	3	0	0	0	—	0	1	40	1	0
,, 2	0	1	0	0	0	0	0	1	3	0	0	1	0	0	0	44	2	0
,, 3	0	2	0	0	0	0	2	0	2	0	2	0	0	0	0	31	0	3
,, 4	0	1	7	2	0	2	5	3	5	0	4	0	0	0	0	2	0	6
July 1	2	1	11	3	0	0	3	0	0	0	0	4	0	0	1	1	0	5
,, 2	0	1	36	3	0	2	2	0	2	1	0	4	0	1	2	0	3	1
,, 3	0	0	6	3	0	96	5	2	0	1	2	71	1	3	23	0	7	1
,, 4	0	0	1	1	2	35	5	0	1	9	2	49	2	32	41	0	2	0
Aug. 1	0	0	0	1	0	50	3	0	0	4	0	9	5		2	0	0	0
,, 2	0	0	0	0	0	17	0	1	0	1	0	4	0	50	1	0	0	0
,, 3	0	0	0	0	0	2	0	2	0	1	2	0	0	18	0	0	0	2
,, 4	0	0	0	0	0	2	0	1	0	0	0	2	0	0	0	0	0	0
Sept. 1	1	0	0	1	2	0	0	2	2	0	1	0	0	0	1	0	0	1
,, 2	0	0	1	0	0	6	0	3	1	0	0	1	0	1	0	2	0	0
,, 3	0	1	1	1	0	8	0	—	1	0	1	—	0	0	—	0	—	1
,, 4	0	1	2	0	2	5	—	—	1	0	0	—	—	0	—	—	—	—
Oct. 1	0	0	1	1	3	2	—	—	—	—	2	—	—	0	—	—	—	—
,, 2	0	2	0	1	0	—	—	—	—	—	—	—	—	—	—	—	—	—
,, 3	0	1	1	0	3	—	—	—	—	—	—	—	—	—	—	—	—	—
,, 4	0	1	0	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—
Total	5	21	72	18	14	227	25	32	27	17	17	148	8	105	72	120	16	21

* = original counts halved to compensate for trap size and colour
† = original counts multiplied by 1.5 to compensate for trap colour
— = no record

TABLE II

Weekly catches of A. fabae *at Rothamsted 1942—1959*

Week		1942*	1943*	1944*	1945*	1946†	1947†	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959
May	1	0	0	0	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—
,,	2	0	0	24	0	2	—	—	—	0	—	—	—	—	—	—	—	0	—
,,	3	10	1	1	0	0	0	6	0	2	—	—	0	—	—	0	—	0	28
,,	4	16	0	13	0	3	0	—	0	5	0	9	0	—	—	0	—	2	14
June	1	9	0	1	0	5	3	—	8	31	1	1	0	—	0	0	26	6	7
,,	2	1	0	2	0	11	2	—	17	11	1	10	1	79	0	0	16	1	7
,,	3	38	0	5	2	3	3	3	35	1	0	9	0	58	0	0	17	1	19
,,	4	24	1	59	6	3	6	2	594	0	0	89	0	0	0	0	21	0	85
July	1	192	4	291	5	23	11	4	846	8	4	29	0	1	0	3	29	1	76
,,	2	92	1	300	12	44	47	13	703	12	20	51	1	74	2	3	30	5	6
,,	3	601	1	304	4	26	192	62	635	4	77	227	1	196	24	2	11	9	20
,,	4	722	0	59	1	21	174	14	493	9	287	42	1	87	393 }	0	43	21	15
Aug.	1	321	0	6	3	14	36	8	3	0	138	6	1	35		1	0	2	45
,,	2	0	0	1	0	0	24	1	0	0	18	1	5	1	738	0	0	7	26
,,	3	18	0	0	1	0	5	0	3	1	19	0	2	6	191	0	0	6	17
,,	4	2	0	0	1	0	0	0	0	0	22	1	1	0	10	0	0	10	8
Sept.	1	0	0	0	0	0	3	0	1	0	2	1	1	0	1	0	0	2	1
,,	2	0	0	0	0	0	8	0	0	0	0	0	1	0	1	0	1	0	1
,,	3	0	0	0	0	0	0	0	0	0	0	0	—	0	0	—	0	—	0
,,	4	0	1	0	0	0	12	—	0	0	0	0	—	—	0	—	—	—	—
Oct.	1	0	0	0	0	0	9	—	0	—	—	0	—	—	0	—	—	—	—
Total		2046	9	1066	35	155	535	113	3338	84	589	476	15	537	1360	9	194	73	375

* = original counts halved to compensate for trap size and colour
† = original counts multiplied by 1.5 to compensate for trap colour
— = no record

N.B. No *A. fabae* was caught during late October

TABLE III

Weekly catches of B. brassicae *at Rothamsted 1942—1959*

Week		1942*	1943*	1944*	1945*	1946†	1947†	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959
May	1	0	0	0	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—
,,	2	0	0	0	0	0	—	—	—	0	—	—	—	—	—	—	—	0	—
,,	3	0	0	0	0	0	0	2	0	0	—	—	0	—	—	0	—	0	0
,,	4	0	0	0	0	0	0	—	0	0	0	1	0	—	0	0	—	0	0
June	1	0	0	0	0	0	0	—	0	2	0	0	0	—	0	0	0	0	0
,,	2	0	2	0	0	0	0	—	0	0	0	0	0	0	0	0	2	0	0
,,	3	1	2	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0
,,	4	0	1	0	0	0	0	1	2	0	0	0	0	0	0	0	2	0	0
July	1	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0	1
,,	2	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
,,	3	0	1	0	0	0	0	0	1	1	0	26	0	0	0	1	0	0	0
,,	4	0	0	0	0	0	0	1	2	0	0	82	0	0	9	1	0	0	1
Aug.	1	25	0	0	0	0	2	0	0	0	0	12	0	0		0	0	0	5
,,	2	0	0	0	1	0	2	0	0	0	0	19	0	0	17	0	0	0	3
,,	3	7	0	0	0	0	0	0	0	0	0	179	1	0	26	0	0	0	4
,,	4	4	0	0	1	0	2	0	1	0	0	64	0	0	3	0	0	0	2
Sept.	1	0	0	0	0	0	6	0	12	0	0	18	1	0	0	0	0	0	1
,,	2	0	1	1	0	0	32	0	20	1	0	8	0	0	1	0	0	0	1
,,	3	0	3	1	0	0	0	0	52	0	0	0	—	0	1	—	0	—	0
,,	4	1	1	1	0	2	12	—	56	0	0	0	—	—	0	—	—	—	—
Oct.	1	0	1	1	0	0	9	—	53	—	—	0	—	—	0	—	—	—	—
,,	2	0	1	0	0	0	—	—	12	—	—	—	—	—	—	—	—	—	—
,,	3	0	1	1	0	0	—	—	1	—	—	—	—	—	—	—	—	—	—
,,	4	0	0	0	0	0	—	—	0	—	—	—	—	—	—	—	—	—	—
Total		38	15	7	2	2	65	4	214	7	0	409	2	0	57	2	5	0	19

* = original counts halved to compensate for trap size and colour (see text)
† = original counts multiplied by 1.5 to compensate for trap colour
— = no record

TABLE IV

Total numbers of aphids trapped at Rothamsted, 1942—59

Year	May	June	July	August	Sept.	Total
1942**	59	291	948	67	15	1380
1943**	317	177	23	11	33	561
1944**	254	206	620	25	32	1137
1945**	37	273	262	27	23	622
1946†	779	3101	3069	680	486	8115
1947†	77*	410	1718	510	395	3110*
1948	859*	506	572	318	32*	2287*
1949	560*	2102	2131	167	161*	4960*
1950	143*	1899	677	352	58	3129*
1951	7*	836	937	399	114	2293*
1952	1592*	954	815	308	78	3747*
1953	70*	150	368	175	29*	792*
1954	—	2137*	1079	715	21*	3952*
1955	31*	265	1140	2041	29	3506*
1956	21*	203	495	149	52*	920*
1957	197*	7731	1823	41	24*	9816*
1958	67	299	321	158	12*	857*
1959	556*	2144	1309	438	150*	4597*

* = incomplete monthly record
— = no record
** = original counts halved to compensate for trap size and colour
† = original counts multiplied by 1.5 to compensate for trap colour.

cae were few during the summer, but *A. fabae* and *B. brassicae,* which overwinter mainly as eggs, were numerous.

1943. Winter and spring were warmer and drier than average and *M. persicae* were few but active early. Many aphids enemies overwintered and probably prevented aphids from becoming numerous during the summer, despite the seemingly favourable weather. *M. persicae* and *B. brassicae* were active again during the autumn.

1944. Winter and spring were drier than usual and the spring migrants were fairly numerous. Both *M. persicae* and *A. fabae* were numerous during July, but not *B. brassicae.* Predators and parasites were again prevalent during the late summer.

1945. A warm and dry spring, but enemies were common and few aphids were trapped.

1946. Another warm winter and spring were followed by a cool, wet summer. Some species were numerous, but few *M. persicae, A. fabae* and *B. brassicae* were trapped.

1947. After an extremely cold winter and cold, wet spring, very few aphids were trapped during May and June. Predators and parasites were scarce and aphids multiplied rapidly during the hot dry weather of July and August. The many *M. persicae* trapped during the first week of the summer migration period did not come from crops near Rothamsted but were blown, possibly from early potatoes in eastern England, bringing with them leaf roll virus and infecting nearly 80%

of the potato plants on the farm. A secondary migration of both *M. persicae* and *B. brassicae* occurred later.

1948. After a warm winter aphids were plentiful in spring, but their enemies also multiplied and populations on the crops remained rather small during the average summer weather.

1949. Another warm winter was followed by a large spring aphid migration. Braconid parasites were particularly numerous when *M. persicae* were colonizing potatoes and prevented a large summer population from developing; when their attacks ceased the aphids increased in number again during August and September. *A. fabae* and *B. brassicae* were extremely numerous during July and September, respectively, when the weather was dry and warm.

1950. A mild winter allowed coccinellid beetles to survive in considerable numbers, and although aphids were active early, their predators again prevented them from becoming numerous.

1951. A cool, wet autumn, winter and spring were followed by an average summer. Aphids were very few early. *A. fabae* became fairly numerous later, *M. persicae* remained few, and no *B. brassicae* was trapped during the summer.

1952. An average spring was followed by a dry, warm summer. Aphids were numerous during the spring, but few *M. persicae* were trapped. *B. brassicae,* however, were more numerous than at any other time during the work.

1953. A cold, wet autumn was followed by an average winter, spring and summer. Aphids were few, but *M. persicae* became numerous during July. On 23 July, 3785 *M. persicae* were counted on 100 potato leaves.

1954. A rather dry winter was followed by a cool, wet summer. Aphids were generally numerous in spring, but few *M. persicae* and no *B. brassicae* were trapped during the summer.

1955. A cold winter and cool, wet spring were followed by a warm, dry summer during which aphids multiplied rapidly, with few enemies, and produced very many migrants of the three species unusually late, during August and September.

1956. Another cold, dry winter and spring were followed by a cool wet summer, and aphids were few. As in 1953, *M. persicae* was an exception, being numerous on potatoes.

1957. Winter and spring were warm, predators and parasites few, and there was the largest early aphid migration recorded. However, enemies multiplied rapidly and summer populations of *M. persicae* and *B. brassicae* were extremely small.

1958. An average winter was followed by a cold, wet spring and cool, wet summer. Aphids of all species were few, and no *B. brassicae* was caught.

1959. A cold winter was followed by a very hot, rather dry summer. Aphids in general were numerous again, and *A. fabae* continued to fly throughout the summer. *B. brassicae* were fairly numerous, but not *M. persicae.*

OTHER SITES

To obtain a comparison with other parts of England, Figs. 1, 2 & 3 show catches from five other sites with those for Rothamsted during the years 1955—57; Table V gives the total numbers of all aphids trapped during the months of June, July and August. The information to be gained from total aphid catches

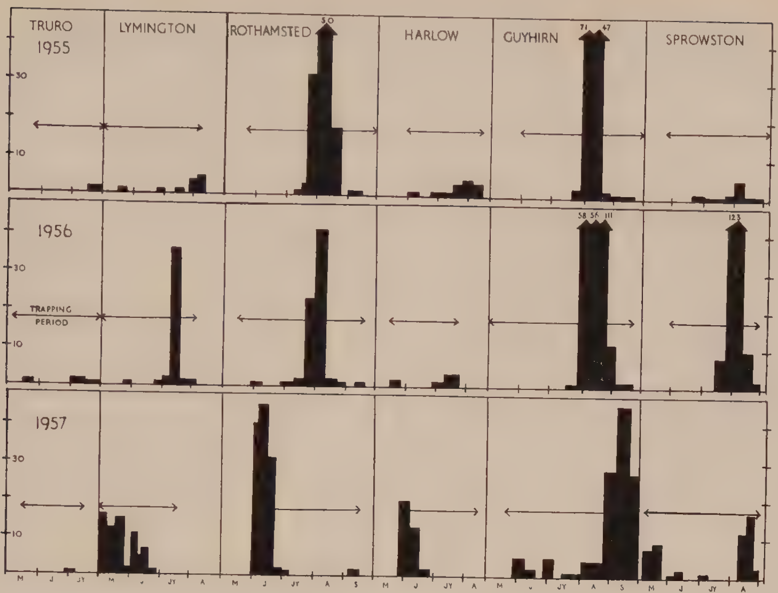

Fig. 1. Weekly catches of *M. persicae* at six sites, 1955—1957.

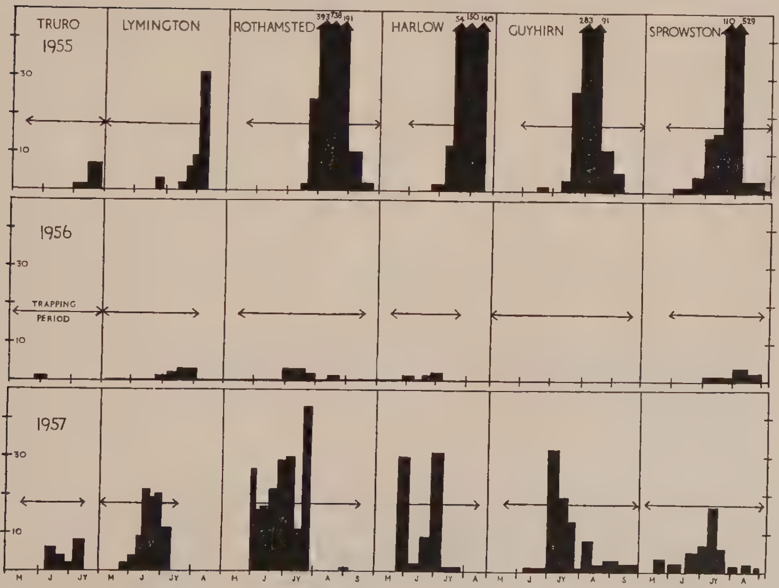

Fig. 2. Weekly catches of *A. fabae* at six sites, 1955—1957.

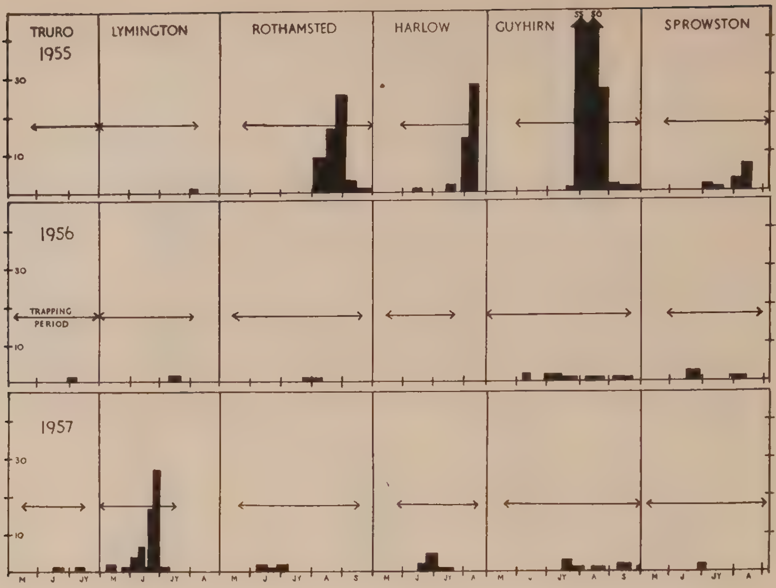

Fig. 3. Weekly catches of *B. brassicae* at six sites, 1955—1957.

TABLE V

Total numbers of aphids trapped at six places during June, July and August, 1955, 1956 and 1957

		Sprowston	Guyhirn	Harlow	Rothamsted	Lymington	Truro
1955	June	550	284	496	265	169	24
	July	360	278	642	1140	384	70
	August	718	1395	403*	2041	102*	—
	Total	1628	1957	1541*	3446	655*	94*
1956	June	75	48	41	203	503	216
	July	189	200	319*	495	457	47
	August	342	276	—	149	37*	—
	Total	606	524	360*	847	997*	263
1957	June	1969	3270	6949	7731	2708	427
	July	647	416	640	1823	35*	52*
	August	114	134	22*	41	—	—
	Total	2730	3820	7611*	9595	2743*	479*

* = incomplete monthly record
— = no record

in different places in different years is limited because one species was sometimes dominant at one site but another species elsewhere, and most aphids caught were often of one species one year but of another the next.

The sites were near Truro, Cornwall; the Efford Experimental Horticulture Station, Lymington, Hants.; Harlow, Essex; Guyhirn, Isle of Ely, and the Norfolk

Agriculture Station, Sprowston, Norfolk. At Lymington and Truro early potato varieties were grown, so trapping usually stopped after the crop was lifted in July; in 1956 trapping at Harlow stopped near the end of July when an experiment was abandoned.

DISCUSSION

The strain of *M. persicae* common in England continues to reproduce viviparously on several crops such as lettuce, brassicas, sugar beet and many weeds during the winter, and most of those found in the spring apparently come from such plants (BROADBENT & HEATHCOTE, 1955). Migrants that reach the potato crops usually make several short flights from plant to plant, depositing a few nymphs on each. These develop into apterous females which produce further generations of mainly wingless females until the maximum infestation is reached, usually towards the end of July. A mainly winged generation then develops and the population decreases as these fly to other hosts, but it may increase again during September. This 'normal' sequence was shown well in 1944, but variations from it are common, and our and other work suggest possible reasons for the variations.

In parts of Europe with very cold winters, where *M. persicae* overwinters mainly as eggs on *Prunus* spp., its numbers on potato crops tend to follow a biennial rhythm of many aphids one season followed by few the next (HILLE RIS LAMBERS, 1955). No rhythm emerges from the catches of *M. persicae* during 18 years at Rothamsted. Four types of trapping pattern are obvious: (1) few or no aphids in spring, more in July and August, and a few later (e.g. 1944); (2) few or none in spring, but many during late July and August (e.g. 1947); (3) relatively few aphids caught at frequent intervals throughout spring and summer (e.g. 1952); and (4) relatively many aphids caught during May and June, but few in July and August (e.g. 1957).

That aphids are adversely affected by wet, cool weather is suggested by the few caught during 1956 and 1958, in contrast to the many during 1947 and 1955, when the summers were warm and sunny. Such correlations are complicated, however, by differences in the initial population after mild or severe winters and by the population of predators and parasites. *M. persicae* were notably few during May and June after the severe winters of 1941—2, 1946—7 and 1954—5 but numerous after the mild ones of 1948—9, 1949—50 and 1956—7. After such mild winters predators or parasites apparently also multiplied readily and prevented large aphid populations from developing on potatoes. The enemies responsible differed in the different years, however; they were braconid parasites in 1949, coccinellids in 1950, and both in 1957. Conversely, the largest summer populations on potatoes occurred during years when aphids and their enemies were very few during the spring — 1947, 1953, 1955 and 1956.

M. persicae never reached such high numbers on the traps as *A. fabae* and *B. brassicae* did in some years. *A. fabae* were by far the most numerous of the three species; only in 1943, 1945, 1953 and 1956 were fewer than 50 caught, in contrast to *B. brassicae,* more than 50 of which were caught only in 1947, 1949, 1952 and 1955, and to *M. persicae* which reached 50 or more only in 1944, 1947, 1953, 1955, 1956 and 1957. Because of the different ways in which aphids overwinter, the differences between their supplies of food at different times, and perhaps the specificity of some enemies, there was no general pattern for the abundance of air-borne aphids of different species. The most aphids trapped during one week were 846 *A. fabae,* 179 *B. brassicae* and 96 *M. persicae,* despite the trap being in the middle of a crop of host plants of *M. persicae.*

Like *M. persicae, B. brassicae* showed no biennial rhythm. Fortunately this very damaging species was seldom very numerous (only in 1949 and 1952). There was, however, a tendency towards a biennial rhythm with *A. fabae*, which overwinters mainly as eggs and seldom as virginogeniae except in the south-west of England. The years with very large catches, 1942, 1944, 1949 and 1955, were followed by years with particularly small ones, but were not all preceded by unusually low years.

The period of maximum catches varied greatly from year to year, but most aphids, and most *A. fabae* in particular, were usually caught during late June and July, whereas *M. persicae* were usually most numerous during late July and *B. brassicae* during August or September.

Most *M. persicae* were often caught at the six places during the same periods. This species was numerous rather late in 1955 and 1956; in 1957, however, most were trapped during May and June, and a late increase occurred only at Guyhirn and Sprowston where they had been relatively few during the spring. Few were caught at Truro in any year.

Annual differences were greater with *A. fabae.* In 1955, when they were exceptionally numerous during July and August, 738 were caught in one week at Rothamsted (the most *M. persicae* caught in any one week at any site during these three years was 123). The next year they were few at all sites and none was trapped at Guyhirn, where they were numerous during 1955. In 1957 they remained active during July, unlike *M. persicae.* Like *A. fabae, B. brassicae* were nowhere numerous during 1956. Very few were caught at Truro in any year, and they were also scarce at Lymington each year from 1954—59, except in 1957 when they were numerous during June and more were caught there than at any other site. In contrast, this species was numerous at Rothamsted, Harlow and Guyhirn in 1955, but scarce in 1957.

Total aphids were fewer usually at Truro and Lymington, near the south coast, and at Guyhirn and Sprowston, nearer the east coast, than in the southern midlands at Harlow and Rothamsted. The reasons for these differences are unknown, for ecological studies on aphids have been few, local and of limited duration. Conditions in England vary so much that trap catches cannot be used to predict the next season's populations, even with a species like *A. fabae* which shows a tendency towards a biennial rhythm. Our results suggest, however, that the following predictions will often be correct: (1) If *A. fabae* are very numerous one year, they will be scarce the next; (2) If *M. persicae* are very numerous during May and June, populations on potatoes will be small during the late summer; (3) If *M. persicae* are scarce during May and June, they will become numerous during late July and August unless the weather is unfavourable.

We are grateful to the many Officers of the National Agricultural Advisory Service who changed trap covers.

ZUSAMMENFASSUNG

1942—1959 IN KARTOFFELFELDERN GEFANGENE GEFLÜGELTE BLATTLÄUSE

In Rothamsted wurden über Kartoffelbeständen 18 Jahre hindurch und anderenorts während kürzerer Zeiträume Aphiden-Klebfallen aufgestellt, um Informationen über die Aktivität von *Myzus persicae* in Beziehung zur Ausbreitung und Bekämpfung von Kartoffelblattroll- und Y-Viren zu erhalten. Die pro Woche gefangenen Zahlen aller Aphiden, von *M. persicae, Aphis fabae* und *Brevicoryne brassicae* werden aufgeführt. Die Höchstzahlen der während einer Woche in Rothamsted gefangenen Aphiden betrugen 846 *A. fabae,* 179 *B. brassicae* und 96 *M. persicae.* Die Bedingungen in England variieren so stark, daß Fallenfänge nicht zur Vorhersage der nächstjährigen Populationen benutzt werden können. Jahresrhythmen sind nicht voraussagbar, aber es bestand bei *A. fabae* eine Tendenz zu geringer Populationsdichte nach einem Jahr, in dem sie hoch gewesen war. *M. persicae* waren im Sommer schwach vertreten, wenn sie und ihre Prädatoren oder Parasiten im Frühling zahlreich gewesen waren, und sie waren im Sommer oft zahlreich, wenn sie im Frühjahr gering an Zahl gewesen waren. Während der meisten Jahre war *A. fabae* häufiger als *M. persicae,* aber *B. brassicae* war nur in zwei Jahren sehr zahlreich. Strenge Winter beeinträchtigten *M. persicae,* welche in England vorwiegend mit aktiven Läusen überwintert, mehr als die beiden anderen Arten, die hauptsächlich als Eier überwintern. Die meisten Blattläuse, und besonders die meisten *A. fabae,* wurden gewöhnlich Ende Juni und im Juli gefangen, während *M. persicae* meist Ende Juli und *B. brassicae* während August und September am häufigsten waren. Die Gesamtaphidenzahlen waren gewöhnlich in der Nähe der Süd- und Ostküste geringer als in den südlichen Midlands. Die Aphiden waren im allgemeinen in warmen, trockenen Sommern zahlreicher als in kühlen, feuchten, aber die Zahlenschwankungen unterschieden sich bei den verschiedenen Arten; die Ursachen dieser Fluktuationen sind vielfältig und noch ungeklärt.

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THE ECOLOGY OF SYMPHYLA
PART III. FACTORS CONTROLLING SOIL DISTRIBUTIONS

by

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Symphylids exhibit complex, seasonal, vertical migrations in soil. The effect of soil moisture content, soil temperature, the presence of plants at the soil surface, feeding cycles, moulting cycles, egg-laying and diurnal rhythms on these migrations were studied. Symphylids migrated in response to changes in soil moisture content and could not survive when soil air was less than 100% R.H. The zone of optimum temperature was 15 to 21° C and survival was limited to the range 2° to 28° C. Food attracted *Scutigerella immaculata* (Newport) in spite of unfavourable soil conditions, but the animals left the surface soil to moult and lay eggs and during non-feeding phases. No diurnal rhythms were found. Most favourable conditions for symphylids included soil temperature in the range 15—21° C, moist soil and growing plants at the surface.

In previous papers (EDWARDS, 1958, 1959) it was shown that symphylids are common soil animals, which, under favourable conditions attain large numbers and exhibit distinct seasonal vertical migration patterns. At some periods of the year they may penetrate deep into the soil and this habit has resulted in speculation about the factors responsible for the soil migrations. Several workers have noted that symphylids are usually absent from the surface layers of soil when it becomes excessively warm and dry, and have expressed opinions as to the cause of these migrations. FILINGER (1931) believed it to be a temperature response, that symphylids aggregated at an optimum temperature of 18°, and that when the surface soil reached temperatures above 21° the animals migrated to lower soil levels. A similar opinion was expressed by WALTON (1930), and KEARNS & WALTON (1932), but these workers believed the optimum temperature to be between 10° and 16° and that if the surface soil became warmer or colder than this symphylids left it.

Other workers such as WYMORE (1924), considered that soil moisture content was the main factor influencing the vertical distribution of symphylids, but neither he nor DUSTAN (1942), who held a similar view, were able to give any conclusive evidence of this. MICHELBACHER (1935), believed that both temperature and moisture influenced the distribution, but came to the conclusion later (1939), that moisture was the more important of the two factors.

In none of this work were there any really careful experimental or ecological observations, and the present study was an attempt to assess the relative importance of moisture, temperature and other factors in controlling these interesting soil migrations. The work involved both field observations and laboratory experiments. The factors which it was considered might possibly influence the distributions are included in the following list:

I. *Environmental Factors*

(a) The moisture content of the soil
(b) The temperature of the soil
(c) The plant cover at the soil surface

II. *Endogenous Factors*

(a) Feeding cycles
(b) Moulting cycles
(c) Egg-laying and care
(d) Diurnal migrations

In addition to these main factors, other changes in environmental conditions such as the carbon dioxide content of the soil air, soil acidity, numbers of predators, and associations with other organisms might be considered to be of some importance. It was difficult to obtain evidence of the effect of these conditions, but such as was obtained, indicated that they were of minor importance, compared with those listed above.

It is probable that the migrations are not usually the result of a single environmental change, but rather a response to a complex of changes, and at different times and places the dominant factor may vary. The aim in the present work is merely to obtain some indication of the relative importance of the different environmental conditions in controlling migrations. To do this the approach has been both in the laboratory and field observations to vary one factor at a time where possible, keeping the others constant and recording its effects. This has also been supplemented by a study of the limiting conditions, i.e. those environmental conditions which symphylids cannot withstand for any length of time. It is realized that the results of any one experiment or observation are inconclusive in themselves, but when considered as a whole certain tendencies emerge and some conclusions can be drawn.

ENVIRONMENTAL FACTORS

The moisture content of the soil

It was possible to find horizontal and vertical gradients of soil moisture in the field, particularly in glasshouse soils. It was noted early in the work, that there were often aggregations of symphylids in the soil close to the walls of glasshouses, and to water taps, both situations where the soil moisture content could be expected to be high. The validity of this assumption was tested by taking a series of soil samples close to a water tap in a glasshouse, after it had been allowed to drip gently for several days. Successive horizontal cores of soil, 6.3 cm diameter and 15 cm deep were removed in four directions at right angles away from the tap. The soil samples were immersed in water and the symphylids which floated out collected and counted. Alongside each core a second similar sample was taken, placed in a container, taken to the laboratory, weighed, oven-dried and weighed again to obtain an estimate of its moisture content. The soil temperature at each sampling point was recorded as each sample was taken. The data of symphylid numbers and soil moisture content for the four directions, were combined and an average obtained (Table I).

TABLE I

Relationship between moisture content and number of symphylids in greenhouse soil

ce ap	6.3	12.7	19.0	25.4	31.7	38.0	44.3	50.8	57.1	63.4	69.7	76.0	82.3	88.6	94.7
re t %	17.0	15.5	15	15.5	15.5	14.5	15.5	15.0	12.5	12.0	11.0	8.0	7.5	8.0	8.5
no. ylids	9.0	5.3	3.0	2.0	5.2	2.5	5.0	3.7	3.0	1.0	1.2	1.0	0	1.0	1.2

Correlation between moisture content and number of symphylids r = 0.794**

There was a highly significant correlation between the soil moisture content, and the numbers of symphylids found per sample, but there was such little difference in the soil temperatures of the different samples (less than 2° C) that it was pointless to attempt to correlate this factor with symphylid numbers.

Further evidence of the influence of soil moisture content on symphylid distribution was obtained in a range of six greenhouses, that invariably had a gradient of moisture in the surface soil from one end of the houses to the other.

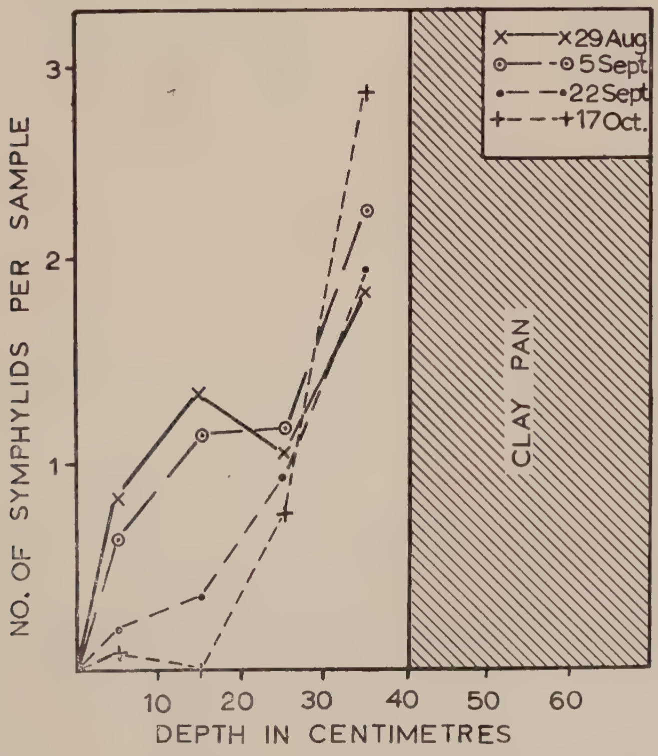

Fig. 1. Changes in vertical distribution of *Symphylella vulgaris* in greenhouse soil during autumn.

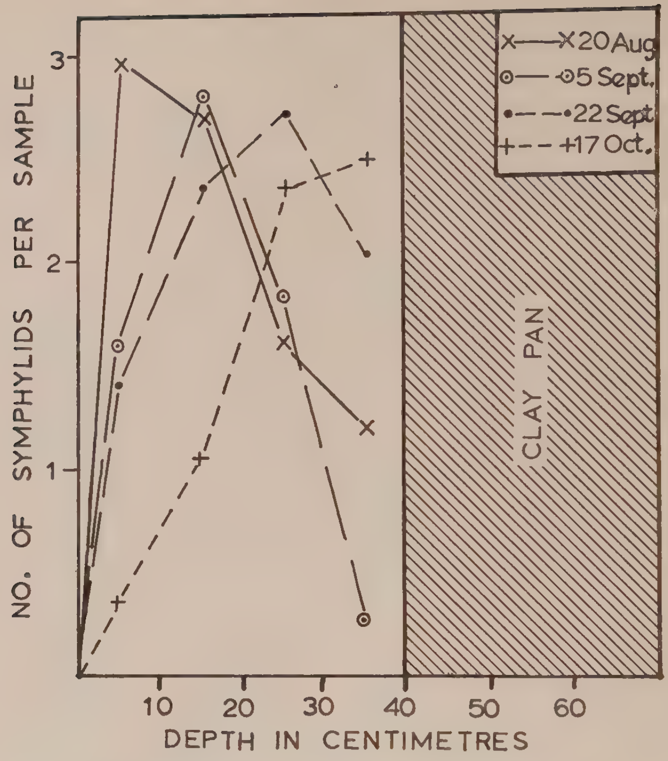

Fig. 2. Changes in vertical distribution of *Scutigerella immaculata* in greenhouse soil during autumn.

In all these greenhouses the total symphylid population at the moist end was estimated at nearly twice that at the dry one.

Frequently nurseries have some greenhouses that are not cropped in the autumn after the tomato crop has been taken, and the gradual drying out of the soil in such a house offered a good opportunity to investigate the effect of this on the vertical symphylid distribution. Soil samples, 6.3 cm diameter, were taken in successive vertical sections, 10 cm deep, and the symphylids extracted by immersion of the samples in water as previously. Six such samples were taken on four dates during the autumn, the symphylid counts for each level averaged, and plotted in Figs. 1 and 2. Temperatures and soil moisture content at different levels in the soil were measured as described in the last section, and are given in Table II.

It can be seen that during the sampling period, the soil temperature at any particular depth fell between 5° and 7° but there was never a marked temperature gradient between surface and subsoil. By contrast moisture conditions changed markedly during the sampling period, the surface soil became very dry, the intermediate layer lost some moisture while the deep soil maintained relatively constant moisture conditions. This gradual drying out of the soil from the surface downwards was reflected in the changes in the vertical distribution of sym-

TABLE II

Soil temperatures and moisture contents in a greenhouse soil during autumn

Sampling Date	Average soil temperature at surface	Average soil temperature 45.6 cm deep	Soil moisture content % 5 cm deep	22.8 cm deep	45.6 cm deep
29th August	19.3°	17.6°	11.8	12.8	12.3
5th September	18.5°	16.3°	8.1	10.9	13.3
22nd September	15.4°	14.0°	5.7	10.6	12.1
17th October	12.3°	12.9°	6.0	7.8	12.5

phylids in the soil (Figs. 1 & 2). As the surface soil became very dry nearly all the symphylids left this region and migrated to moister soil below.

This reaction to moisture content was confirmed in the laboratory by use of a gradient of moisture content. A copper-lined trough, 75 cm long and 7.5 cm wide was filled to a depth of 1.25 cm with sandy-loam soil that had been oven-dried, and then sieved to a particle size of two to three millimetres. Water in graded quantities was sprayed on to the soil so as to obtain a gradient of soil moisture content along the length of the box. After a period for equilibrium to be reached one hundred symphylids (*Scutigerella immaculata*) were randomly distributed along the box. After twenty-four hours the soil was removed from the box in 15 cm lengths and the symphylids recovered by hand sorting. Samples of soil from each section were weighed, oven-dried, and reweighed to obtain an estimate of the soil moisture content. The distribution pattern summarized in Table III is the average for three experiments.

TABLE III

Distribution of symphylids a soil moisture content gradient

Section No.	1	2	3	4	5
Avge. soil moisture content	4.8%	6.3%	9.4%	11.2%	16.7%
Avge. no. of symphylids present	0	6.3	27.0	37.3	26.0

All these observations have shown that symphylids will react to a gradient of soil moisture content. It is however important to distinguish reactions to moisture content and those to relative humidity of soil air. For the greater part of the year the soil air may be at a relative humidity of 100% and only when the soil becomes very dry does it fall below this.

In the laboratory, the reaction of symphylids to relative humidity was measured in a humidity choice apparatus modified from that described by GUNN (1936). The apparatus was much smaller and the alternative humidities on the two sides were obtained by dilutions of sulphuric acid solutions. In each experiment 100 symphylids were placed in the apparatus and their distribution after two hours determined. Results from experiments giving choices which involved low humidity on either side of the apparatus were erratic since there was considerable mortality at these low levels.

The intensity of reaction to humidity was estimated by use of the formula given by GUNN & CONWAY (1938):

$$\text{Excess Percentage Ratio} = \frac{100\ (\text{No. of animals on humid side} - \text{No. of animals on dry side})}{\text{No. of animals on humid side} + \text{No. of animals on dry side}}$$

An excess percentage ratio of 0% indicates no reaction to humidity and one of 100% that all animals were found on wet side of chamber.

The results of five experiments at higher humidities are summarized in Table IV.

TABLE IV

Humidity choice reactions of symphylids

High humidity	100	100	100	100
Low humidity	79	84	89	95
Excess % ratio	91.5	60.0	39.8	—3.4

Least significant excess % ratio : 14.2 at 0.05 level
23.4 at 0.01 level

The results indicated that symphylids were strongly hygrotaxic under the conditions of these experiments, and could distinguish a difference of 10% in relative humidities when given a choice between two different high humidities. The results were confirmed in a small humidity gradient apparatus only a few inches long (MADGE, 1961), that gave a full range of humidities from 0 to 100%. Here with the exception of a few individuals that became immobilized and eventually died at the dry end, all the symphylids moved to the moist end after about half an hour, although they continued to move about over the surface of the sintered glass that formed the floor of the chamber, and even made occasional excursions into the drier half.

The survival of symphylids at different humidities was investigated in small

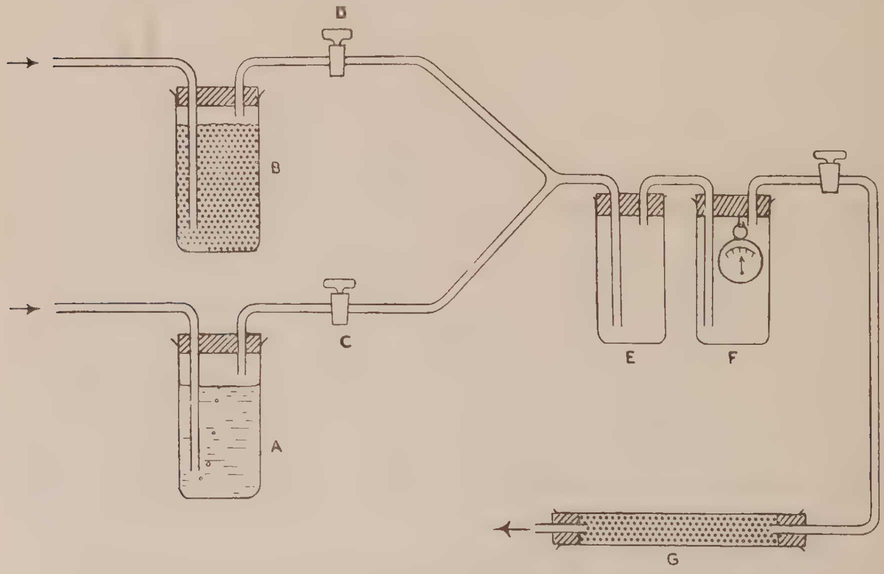

Fig. 3. Apparatus for determining relationship between soil moisture content and relative humidity of soil air.

humidity chambers which consisted of small glass jars with pieces of fine nylon mesh supported half way up. A saturated solution of an appropriate chemical was placed in the bottom of each jar and small paper hygrometers were suspended above the mesh. After equilibrium had been reached ten symphylids were introduced into each chamber through a small hole in the lid and mortality counts made at intervals. Mortality at the lower humidities was very rapid, and only when the relative humidity was 100% did symphylids survive for as long as twenty four hours. These tests were made under unnatural conditions, but were confirmed by another experiment which also related the humidity of soil air to soil moisture content. The apparatus which is shown in Fig. 3, gave a stream of air of known constant relative humidity which was passed through oven-dried soil until it reached equilibrium. Compressed air was passed into the apparatus and divided into two pathways. In one of these (A), the air bubbled through water, and in the other (B), passed through calcium chloride to extract all moisture. The two air streams were controlled by taps C and D and passed into the vessel E, where they were mixed in proportions appropriate to the required humidity. The flow continued through another vessel F, where the humidity was read on a suspended paper hygrometer. Finally the air passed into the tube G, which contained the soil sample. It was essential that each experiment was carried out at a constant temperature, since small differences of temperature resulted in considerable changes in the humidity of air passed through the soil. In each experiment a weighed quantity of freshly oven-dried soil, sieved to a particle size of between two and three millimetres, was placed in the tube and one hundred symphylids were introduced. Air of the required constant humidity was passed through the soil for twenty four hours, when the soil was removed, and the symphylids extracted by handsorting. Finally the soil was reweighed in order to determine the moisture that had been taken up from the air. Similar experiments were done for a range of constant humidities.

The results of the first experiment were unsatisfactory since 100% mortality occurred at all relative humidities when air was passed through the apparatus in the way described. In a second experiment the symphylids were introduced after the air had been passed through the soil for twenty four hours, and then turned off; the results of this experiment agreed with those obtained previously over chemical solutions, i.e. appreciable survival for more than twenty four hours only occurred when the relative humidity of the air that had been passed through the soil had been 100%.

This experiment also provided a means of determining the relationship between the moisture content of the soil, and the humidity that existed in the soil air between the soil particles. If a graph is drawn plotting the humidity of air passed through the soil against soil moisture content (Fig. 4) it can be seen that there is a certain limiting soil moisture content, above which the soil air is always at 100% relative humidity. If the moisture content falls below this value the soil air has a relative humidity less than 100%, and this may be sufficient to drive the symphylids into moister soil. It can be seen that for the particular soil tested the soil air fell below 100% relative humidity at soil moisture contents below 6%.

To summarize the results obtained in this section, it appears that symphylids react strongly to soil moisture content, and even more strongly when the moisture con-

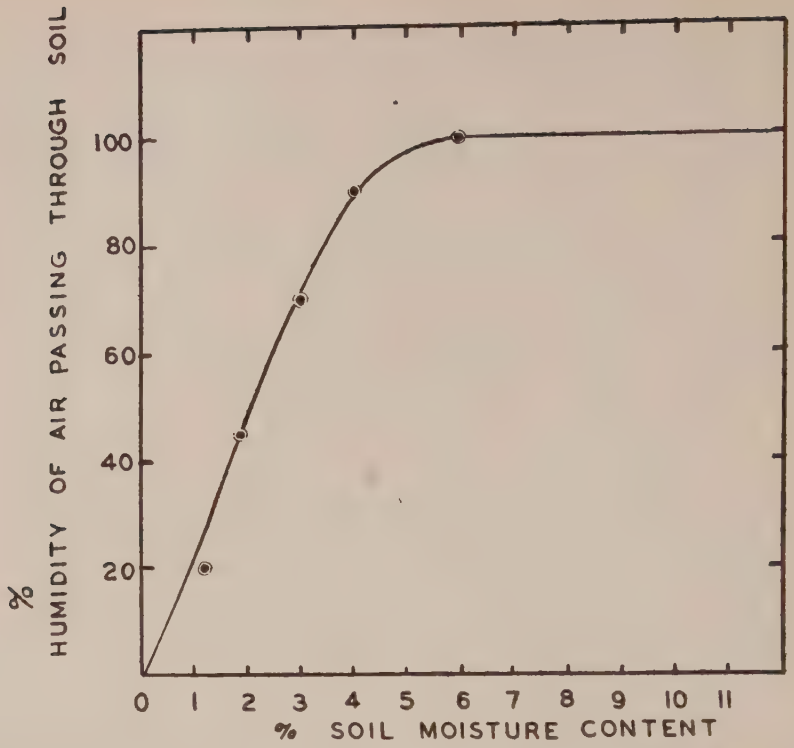

Fig. 4. Relationship between soil moisture content and relative humidity of soil air for a loam soil.

tent falls to a level at which the soil air has a relative humidity of less than 100%, under which conditions they cannot survive for long.

The temperature of the soil

It was possible to find gradients of soil moisture content in the field at constant temperatures. It was not possible however to find soil temperature gradients independent of soil moisture. Attempts to produce these artificially in the field met with little success, so that observations on the effect of temperature on symphylids were almost entirely confined to laboratory experiments. Even in the laboratory similar difficulties were encountered.

Field observations indicated that symphylids were relatively tolerant of wide temperature changes. Large numbers were found outdoors, actively feeding on plant roots, at soil temperatures as low as 2° C, although it should be noted that in previous studies of the seasonal distribution of two species (EDWARDS, 1959), they usually leave the surface soil during the colder winter months. High surface temperatures during the summer months also resulted in some migration from the surface soil, but this may have been in response to the drying out which accompanied these higher temperatures. Where susceptible plants were present in the surface soil symphylids could be found even when the soil temperatures were excessively high or low.

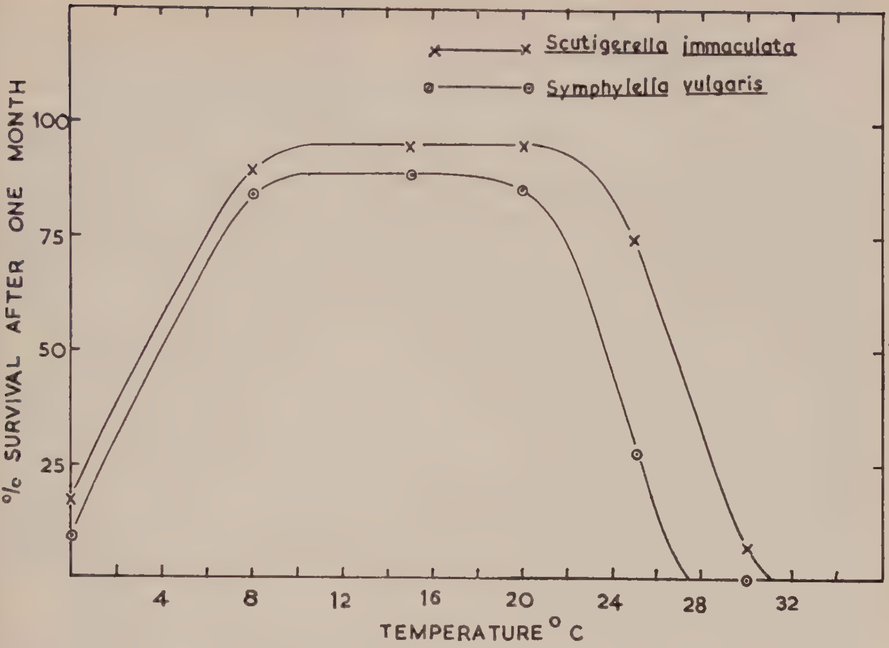

Fig. 5. Survival times of symphylids at different temperatures.

The temperature tolerance of symphylids was investigated in laboratory experiments, which consisted of regular examination of cultures of symphylids kept at constant temperatures. Unfortunately the range of constant temperatures available was limited, but nevertheless the data summarized in Fig. 5 showed that both species of symphylid could survive at a wide range of temperatures for quite considerable periods. Even at 0° C there was some survival, although the animals were inactive at this temperature. Certainly the symphylids were able to survive at all temperatures that commonly occur under field conditions, except in soil very close to the surface. Further there was some evidence of adaptation to low or high temperatures. Cultures of symphylids were kept at 8°, 15° and 20° for three months and then exposed to 0° for a month. Of these animals 28% of those bred at 8° survived, but only 17.5% of those at 15° and 19.0% of those reared at 20° were still alive at the end of the month. This is evidence of acclimatization to low temperature similar to that described for several insects by MELLANBY (1939).

In the course of these experiments it was observed that temperature strongly influenced the moulting rate, and since there is evidence that moulting was associated with migrations, this secondary effect of temperature is considered in more detail later.

The behaviour of symphylids in temperature gradients was investigated in the laboratory. In early experiments a temperature gradient apparatus based on that described by GUNN (1928) was used, but difficulty was encountered in the gradient of humidity that accompanied the temperature gradient. In this apparatus the symphylids always migrated to the cold end of the trough which was also the humid end. Attempts to maintain a high humidity along the whole length of the trough by water, damp soil or damp filter paper met with little success.

These experiments were repeated in a much smaller gradient designed by MADGE (1961), which produced very much less variation in relative humidity along the trough when damp filter paper was placed on its floor. Further the variations in temperature and humidity had been worked out in some detail. This apparatus consisted of a small glass trough 22 cm long, and 4 cm wide, with a copper bar as wide as the trough and 0.2 cm thick on its floor. Either end of the copper bar was inserted into a lagged constant temperature trough, and on its surface was laid a piece of moist filter paper intended to maintain the humidity as high as possible along its length. Temperature was measured by means of a small thermistor which could be inserted at different points along the gradient. At one end the container was packed with ice and salt, and warm water was placed into the other. After equilibrium was reached and a temperature established, twenty-five symphylids (*Scutigerella immaculata*) were introduced into the apparatus, and counts were made of the distribution at ten minute intervals. From time to time hot or cold water was added to the warm end to change the range of temperature along the gradient. This was in order to obviate any possible aggregation of the symphylids at some particular position along the gradient in response to some static factor other than temperature.

The results of a number of counts summarized in Table V, show that there was a highly significant aggregation of symphylids between 14.9° and 18.6°. The value for aggregation between 19.2°—20.7° was also significantly above that for 21.2°—22.8°. We may conclude that the preferred temperature range for symphylids lies in the region 15° C to 21°. In this apparatus with moist filter paper on the floor there was much less variation in relative humidity, and in fact MADGE (1961), has shown that the air is saturated in the warm half of the apparatus but falls off somewhat at the cold end to about 80% humidity. To nullify the effect of this, different temperature ranges were used, and there was still aggregation in the same temperature range, even when this was situated within the low humidity part of the gradient. Hence it seems likely that under the conditions of these experiments the effect of temperature overcame the effect of humidity.

TABLE V

Distribution of symphylids in temperature gradients

Thermistor reading	48 - 51	52 - 55	56 - 59	60 - 63	64 - 67	68 - 71	72 - 75	76 - 7
Temperature ° C	25-23.4	22.8-21.2	20.7-19.2	18.6-16.9	16.4-14 9	14.4-12.8	12.2-10.7	10.2-8.
Mean no. of symphylids per count	0.55	0.36	2.27	6 53	10.36	1 0	1.82	1.2

Least significant difference 0 05 - 1.80

Least significant difference 0.01 - 2.44

The range of temperature preference demonstrated in these experiments shows some relationship to the range of temperature at which activity is greatest. Below

5° C the animals were almost completely inactive, between 5° and 10° they were very sluggish but between 15° and 20° they reached a peak of activity which did not increase appreciably even at higher temperatures.

The plant cover at the soil surface

Some species of symphylid are economic pests, so that it might be expected that they would be attracted to roots of susceptible plants. Of the two species studied, one, *S. immaculata*, is such a pest, and the other, *S. vulgaris*, is not. In a study of an infestation of strawberry plants where both species were present, the influence of the plants on the vertical distribution of the two species was very marked.

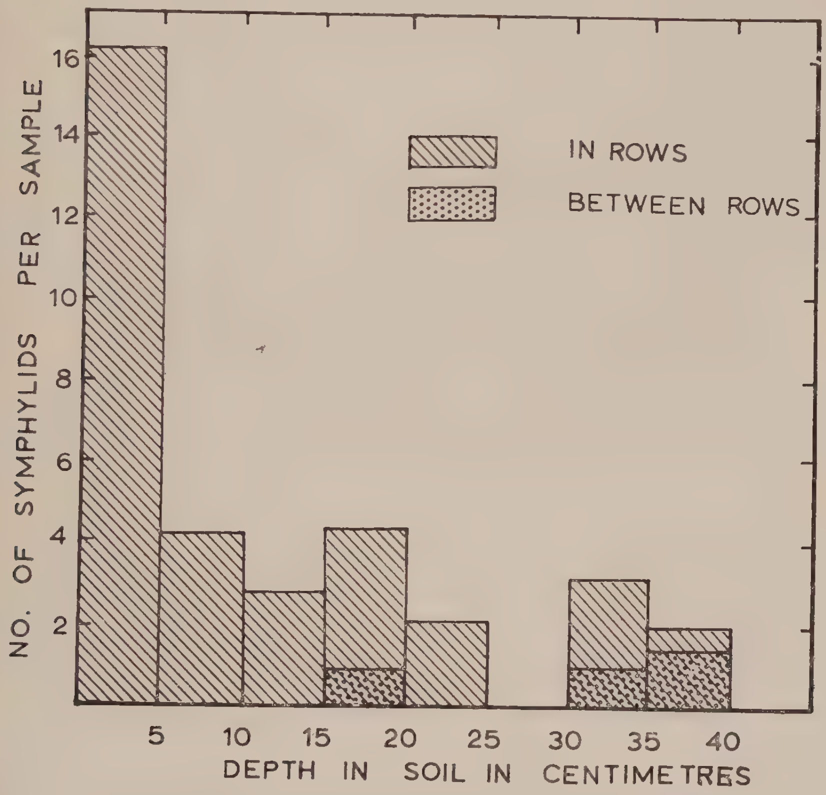

Fig. 6. Distribution of *Scutigerella immaculata* in relation to strawberry plants.

The vertical distribution of the two species in relation to the plants was estimated from a series of twenty, 5 cm diameter, cores taken in 5 cm, vertical, layers down to the sub-soil, from the rows of plants; and a similar series taken in between the rows. The results of the sampling were averaged for each level and the results plotted in Figures 6 and 7. The surface numbers of *S. immaculata* were very high in the rows but not between the rows, and it was only in the deeper samples that any were found between the rows. This is easily explained in terms of the presence of food in the rows, but the higher numbers of *Symphylella vulgaris*

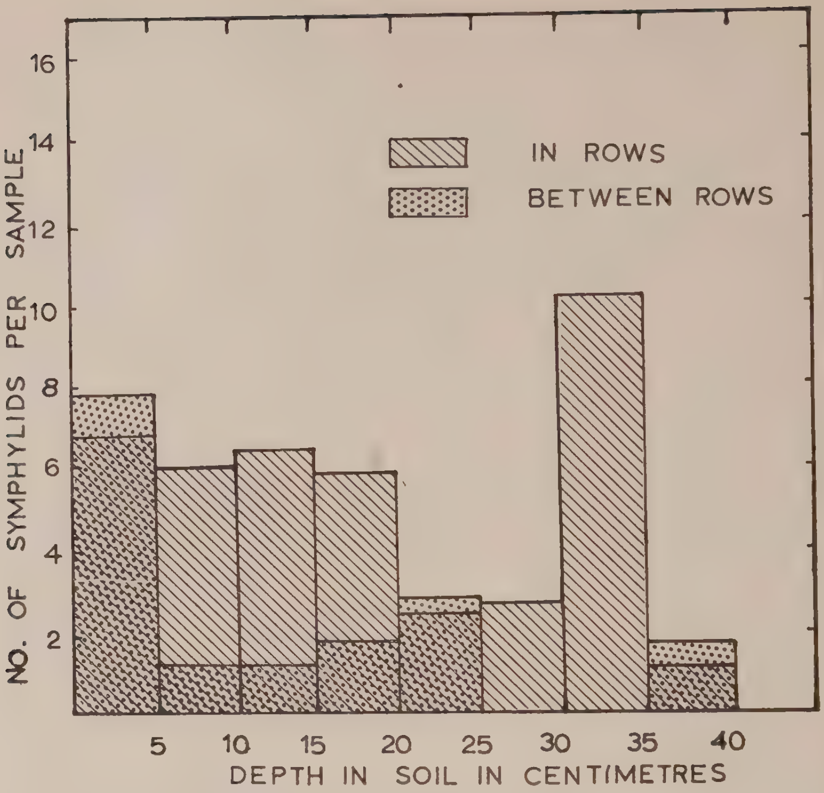

Fig. 7. Distribution of *Symphylella vulgaris* in relation to strawberry plants.

Hansen found in the rows, although not nearly so pronounced as seen in the other species, indicate that there is some attraction to the plants. This may not mean that they were feeding on healthy roots but possibly they were attracted to decaying roots or root exudate. Similar distributions were found under both tomato and lettuce plants, and it was also noted that if tomato, lettuce or other seedlings were transplanted into soil in which specimens of *S. immaculata* were reasonably evenly distributed, the symphylids became concentrated around the roots within a period of five days.

The attraction of symphylids to roots was confirmed by burying tomato roots at various positions in the soil and examining them after several days. In each case the symphylids were heavily concentrated around the roots. The horizontal movement of *S. immaculata* was investigated by surrounding some newly transplanted tomato plants with boards buried to a depth of 45 cm. One week after planting out, these plants were dug up and the symphylids removed by flotation on water, and the same number of plants without boards also examined. There was no apparent difference between the numbers of symphylids under the two sets of plant, which indicated that they had been principally infested by symphylids which had migrated up from a depth of more than 45 cm, and that there was a constant flux of individuals from the lower soil to the upper, and back. The rapid rate at which plants become infested suggests that some form of attraction takes place, possibly along a gradient of root exudate, as is the case in wireworms. The field observations were confirmed in the laboratory by planting tomato seedlings at one end of a long box, and regularly applying root exudate to the other. After a week the distribution was examined and most symphylids were found under the tomato seedlings, but nevertheless there were considerably more at the end that had been treated with root exudate, than in the centre portion which had only been treated with water.

To confirm whether symphylids move over the surface of the soil, seedlings were planted at one end of a box which had a partition down the middle, projecting 1.25 cm above the soil surface. Symphylids were introduced into the soil at the end without plants, and again examined after a period, this time two weeks. Large numbers were present around the roots, and these must have made their way over the barriers to the plants.

Finally a long box was filled with oven-dried soil, and one end was dampened thoroughly and infested with symphylids, while at the other some healthy tomato roots were buried. After two days there were more symphylids in the end of the box which contained the roots, although in a similar experiment without the roots described earlier (Table IV) no symphylids were found in the end of the box with dry soil. It appeared that symphylids were still attracted to food even when soil conditions were adverse.

ENDOGENOUS FACTORS

It is much more difficult to assess the effects of endogenous factors experimentally than the effects of environmental ones. The approach in the present study has been principally to make field observations and where possible to confirm these in the laboratory.

Feeding cycles ,

Some symphylids such as *Scutigerella immaculata* feed on a wide range of food material, not only yeasts, bacteria, fungi, and live and dead plant material, but also on other dead soil animals. Other species are much more limited in food range and do not feed on living plant tissues to any extent. It might be expected that the soil distribution of the former species would be much more influenced by the presence of growing plants at the surface, than is the latter, and it was shown in the last section that this is the case. It was noted however, that in laboratory cultures of *S. immaculata* with abundant food, there were periods when no feeding occurred, and this was further investigated.

Individual specimens of *S. immaculata* were kept in small separate culture containers (EDWARDS, 1955), in the bottom of which was a layer of sterilized plaster of Paris. Each symphylid was daily supplied with a fresh weighed piece of lettuce leaf, one centimetre square and containing no large veins, and this was reweighed and renewed after twenty four hours. Similar pieces were added to control chambers containing no symphylids, and all cultures were kept in an incubator at 20°. The loss of weight of the lettuce, corrected for gain or loss of weight of that in the control, gave an estimate of the amount of feeding that occurred. When the results were plotted they gave feeding curves such as those shown in Fig. 8.

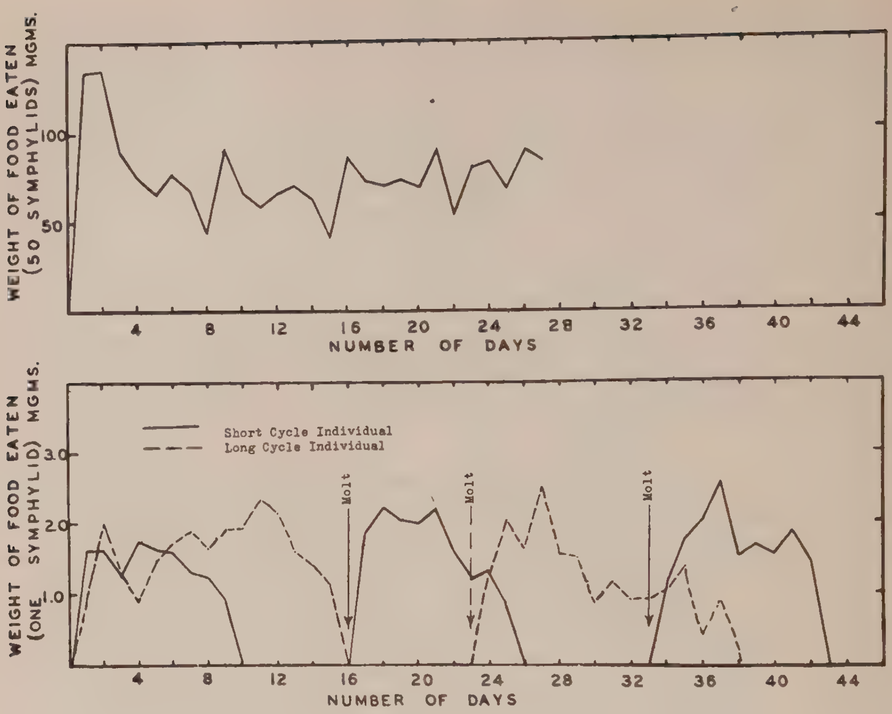

Fig. 8. Moulting and feeding cycles of *Scutigerella immaculata.*

Prior to the experiments the symphylids were starved, and during the first twenty-four hours of the experiment fed extremely voraciously, so that sometimes as much as fifteen times its own weight was consumed by a single individual in twenty-four hours. Feeding then fell to a somewhat lower level until some five to ten days prior to moulting. During this premoult period no feeding occurred at all. There were considerable differences between individuals in the length of the feeding and non-feeding phases, but individual symphylids were consistent in their feeding and moulting pattern. With a particular symphylid if there was a long period between moults initially, then this pattern was maintained indefinitely under constant environmental conditions, and the same was true of individuals with a short period between moults. This feature was probably of genetic origin since environmental changes only emphasized the basic pattern. The moulting rate increased considerably with rise in temperature.

It is probable that other species of symphylids have similar feeding cycles, but it was much more difficult to demonstrate this. It is likely that the importance of feeding phases in relation to soil migrations, is much less with those species that do not feed on living plant material, since plants are only present at particular times in the year. In the case of *S. immaculata* it was possible to investigate whether the animals associated with plant root systems in the field were in a feeding phase. Soil samples were taken in a heavily infested greenhouse planted with tomato plants. From these one hundred symphylids were collected from each of three soil strata, 0—15 cm, 15 cm—30 cm and 30 cm—45 cm, both close to the plants and in open soil between the rows. Samples were also taken from amongst the roots of plants. Specimens were taken back to the laboratory and confined in individual cultures together with small pieces of lettuce leaf, and examined at intervals to determine whether feeding occurred. Results are given in Table VI.

TABLE VI

Percentages of symphylids found in feeding phase

	Around roots	0—15 cm	15—30 cm	30—45 cm
Under plants	97%	88%	33%	56%
Open soil	0	72%	61%	48%

Practically all the symphylids found around the plant root systems were in a feeding phase, whereas in other parts of the soil one third to one half of the symphylids were in a non-feeding phase. This is good evidence that symphylids leave the root system when not actually feeding. This was confirmed by observations in the laboratory of soil cultures of symphylids kept in deep glass vessels. Symphylids feeding on lettuce leaves near the surface usually returned to deeper layers after feeding.

Moulting Cycles

As was shown in the last section moulting is intimately linked with the feeding cycles, and there is evidence that many symphylids leave the plant root system when not feeding. Soil was intensively sampled for cast skins, which were floated out of the soil on water. Results obtained in heavily infested soil are given in Table VII, but are only based on a single set of samples taken in March.

TABLE VII

Depth distribution of symphylid cast skins in soil

Depth of samples	0—15 cm	15—30 cm	30—45 cm
No. of cast skins in 20 samples (6.5 cm diam., 15 cm deep)	11	68	13

Observations were also made on laboratory cultures by floating out cast skins in layers of soil taken at different depths. Again nearly all the cast skins were found in the deep samples.

Egg-laying

In laboratory cultures, eggs were nearly always laid near the bottom of the large culture jars in clusters, and were commonly closely guarded by an adult symphylid. Satisfactory hatching appeared to be dependent on parental care, since when the adult symphylid was removed the great majority of eggs failed to hatch.

Eggs were not common enough in soil samples to obtain exact numerical data on their vertical distribution in the soil, but it was noted that eggs never appeared to be laid within 5 cm of the soil surface, and were much more common in the lower soil layers, particularly in the range 5—15 cm. It is possible that in spring when egg-laying is at its height, there is some aggregation in this zone, but this factor is probably of minor importance in affecting the vertical distribution.

Diurnal rhythms

Friedel (1928) indicated that he could collect more specimens at the surface under stones in the morning than in the afternoon. It was suspected in view of this that there might be some form of diurnal migration. To investigate this samples were taken at various times throughout the same day from a small area of soil, and specimens were extracted by water flotation. The results did not show any significant variation in vertical distribution through the day.

CONCLUSIONS

In this study most emphasis has been placed on the effect of environmental changes on the complex vertical migrations of symphylids. This is only because these are factors which can be relatively easily controlled and their effects observed. Nevertheless it is quite impossible to explain these migrations purely in terms of these factors. Endogenous factors which need considerably more study than has been possible here, must exert a very large influence in initiating the movement of symphylids from depths as great as 1.8 m, where the moisture and temperature conditions must be relatively constant through the year. Nevertheless, nearer to the surface it seems that changes in environmental conditions must play a very large part in influencing migrations.

The effects of the environmental conditions such as temperature and soil moisture content may take several forms. There may be actual preference for a particular range of temperature or moisture, migrations may occur away from conditions where survival is impossible or the environmental conditions may result in secondary effects which influence the migrations.

In the case of moisture content there appeared from the field observations, to be an attraction to soils with a higher moisture content, which was demonstrated in the field away from a dripping tap and in the laboratory in a gradient of soil moisture content. This appears to be in addition to the actual avoidance of soil in which the moisture content has fallen to a level such that the relative humidity between the soil particles has fallen below 100%. It was demonstrated in the laboratory that symphylids will move away from humidities below this level. The critical level of moisture content below which the humidity of the soil air is less than 100% varies considerably with the soil type. It may be as low as 3% in a pure sand, while in a very heavy soil values as high as 20—30% may be obtained. Such values do occur in surface soil outdoors in hot dry weather between May and October and even down to a depth of 60 cm (Black, Corney & Jacobs, 1958). Greenhouse soils in autumn are particularly susceptible to drying out and in such cases this is almost certainly responsible for the migration of symphylids to lower levels. But effects such as these cannot completely explain the penetration of symphylids to the deeper soil levels. Here the influence of water in the soil must be reaction to soil moisture content itself and the mechanism by which this preference operates is more difficult to determine. It is also very interesting that symphylids appear to have a positive reaction to high soil moisture content both in a horizontal direction and a vertical one. It appears that in certain conditions hygrotaxis was dominant over geotaxis, a comparatively rare phenomenon.

In the same way there was a preference for a particular temperature range, 15°—21°, temperatures which commonly occur in the surface soil during spring and autumn, and at depths down to 60 cm in summer. In summer close to the surface they may be well above this level and in winter considerably below. In fact at certain times of the year the surface soil temperature almost certainly may be completely limiting and reach levels below 2° and above 28° where survival of the symphylids may be seriously affected. In studies by BLACK, CORNEY & JACOBS, (1958) the soil temperature even at a depth of 30 cm went down to almost 0° and up to over 20° every year between 1952 and 1955, and the surface variations must have been very much greater than this. Sudden large changes in soil temperature such as occur in surface soil on a hot sunny day may have more effect on migrations than gradual changes over weeks or months since there was evidence of acclimatization to low temperatures and the same probably holds good for high ones. Even at a depth of 1.5 m the soil temperature went down to about 5°. Temperature may also have secondary effects on migrations in the form of increased activity, more rapid feeding and more frequent moulting at the higher temperatures.

The effects of plants in the surface soil is somewhat different for the two species of symphylid studied, but in the case of the phytophagous species the influence is very great, and will attract symphylids into zones where temperature and moisture conditions may be far from optimal. As was seen in previous work the presence of plants at the surface modifies the depth distribution pattern for *S. immaculata* very considerably (EDWARDS, 1959).

Superimposed on these migrations in response to changing environmental conditions, are those in response to feeding and moulting cycles, egg-laying and other endogenous factors.

These observations make it much easier to understand the complex seasonal changes in vertical distribution described in the previous paper in this series. It seems likely that temperature plays a major part in determining the vertical distribution, even more important than moisture, but they are so intimately linked that it is very difficult to assess the relative importance. The most valuable conclusions which can be reached, is that if there are plants at the soil surface, a soil temperature in the range 15—21° and a moist soil, a large percentage of the symphylids present is likely to be concentrated in the surface soil. From the economic aspect this conclusion is important since it defines the optimum conditions to apply treatments for the control of symphylids.

ZUSAMMENFASSUNG

DIE ÖKOLOGIE DER SYMPHYLIDEN. TEIL III. FAKTOREN, WELCHE DIE VERTEILUNG IM BODEN REGELN

Die Symphyliden zeigen komplizierte, jahreszeitliche Vertikalwanderungen im Boden. Es wurde ein Versuch unternommen, die relative Bedeutung der verschiedenen exogenen und endogenen Faktoren abzuschätzen, welche diese Wanderungen beherrschen. Die Hauptfaktoren, die im Laboratorium und durch Freilandbeobachtungen untersucht wurden, waren Feuchtigkeitsgehalt des Bodens, Bodentemperatur, Anwesenheit von Pflanzen auf der Bodenoberfläche, Fraßzyklen, Häutungsfolgen, Eiablage- und Tagesrhythmen. Es konnte in entsprechenden Feld- und Laborproben nachgewiesen werden, daß die Verteilung der Symphyliden zum Feuchtigkeitsgehalt des Bodens in Beziehung steht. Bodenfeuchtigkeitsgehalte, die

einer relativen Feuchtigkeit der Bodenluft von weniger als 100% entsprachen, ergaben eine Abwanderung der Symphyliden in andere Bodenschichten. Das Überleben der Symphyliden über nennenswerte Zeiträume erfolgte nur bei einer relativen Feuchtigkeit vom 100% in der umgebenden Luft. Versuche zur Bestimmung des Temperaturgradienten zeigten, daß der optimale Temperaturbereich für Symphyliden zwischen 15 und 21° C liegt und daß die Lebensfähigkeit von Temperaturen unter 2° und über 28° begrenzt wird. *Scutigerella immaculata*, eine phytophage Art, konnte eine Zone besiedeln, wo die Bodenbedingungen für die Ernährung ungünstig sind. Sie zeigte außerdem Fraß- und Hungerphasen, indem sie während der Hungerphasen in tiefere Bodenschichten wandert, um sich zu häuten. Die Eier wurden gewöhnlich in der Bodenschicht zwischen 5 und 15 cm abgelegt. Tagesrhythmen ließen sich nicht finden. Es wurde gefolgert, daß die günstigsten Bedingungen für eine hohe Anzahl von Symphyliden an der Bodenoberfläche in der Anwesenheit von Pflanzen an der Bodenoberfläche, einer Bodentemperatur im Bereich von 15—21° und feuchtem Boden bestehen.

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SOME ASPECTS OF THE PHYSIOLOGY AND BEHAVIOUR OF *GALEODES ARABS*

by

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Galeodes arabs is shown by aktograph experiments to be nocturnal in habit especially in the hot season. It has a low rate of water-loss by transpiration in dry air. Behaviour responses to enemies and prey are classified as alertness, threat, high intensity threat (accompanied by stridulation) leading to attack or flight (often followed by displacement sand-digging). Feeding habits are described.

The camel-spider, *Galeodes arabs* C. L. Koch, is a characteristic inhabitant of the desert around Khartoum. It is nocturnal in habit and spends the day in a deep burrow, the mouth of which is often closed with a plug of dead leaves. Not much is known of the biology of Solifugae, but the available information has recently been summarised (Cloudsley-Thompson, 1958; 1961b). This paper describes the results of experiments on the diurnal rhythm of activity, water-relations, aggressive behaviour and feeding habits of the species. It forms part of a programme of research designed to investigate the adaptations of desert arthropods to life under extreme conditions of aridity and heat.

The camel-spiders were kept separately in perspex or glass vivaria lined with sand. They were fed on cockroaches, grasshoppers or scorpions.

RHYTHM OF ACTIVITY

In the wild, camel-spiders spend most of the daytime in their burrows and only emerge after dark when they are sometimes attracted to light and enter houses on the outskirts of Khartoum. They were inactive in captivity too during the day, but at night would roam about, biting the sides of their cages.

The diurnal rhythm was investigated by means of aktograph apparatus (Cloudsley-Thompson, 1961a). Fig. 1 shows the aktograph record of an immature specimen from the time of moulting. It will be seen that no activity took place for three or four days after ecdysis, after which the normal 24-hour periodicity was resumed. Similar results have been obtained with other individuals in various stages of development, including adults. Evidence has also been obtained indicating that the diurnal rhythm of activity may be more rigid in the hot season than in winter. Some records made in December and January, for example, showed a certain degree of day-time movement, which was not to be seen in records made during the summer.

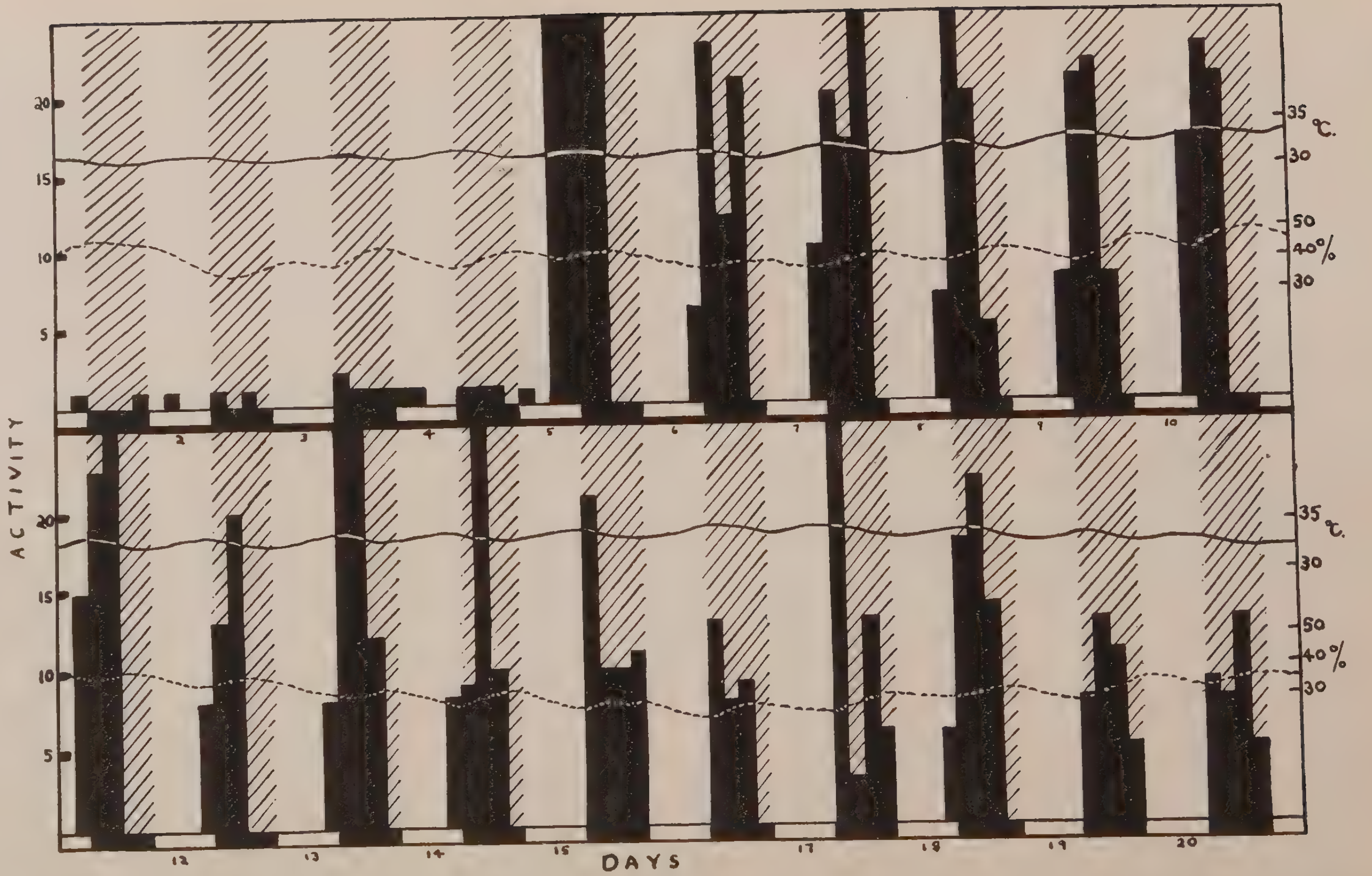


Fig. 1. Analysis of aktograph records showing periodicity of movement and rest in *Galeodes arabs*. Ordinates: left; activity; right; temperature (solid line) and relative humidity (broken line). Black strips: 12 hr periods from 18.00 to 06.00 hrs: oblique lined areas: periods of

WATER-RELATIONS

In order to test the ability of *G. arabs* to conserve moisture, a large female camel-spider was fed with a cockroach on 27 July 1960. It was then anaesthetised with ether, weighed and placed in a desiccator over anhydrous calcium chloride at a room temperature of 33° ± 2° C. It was anaesthetised and re-weighed at intervals of 72 hours until its death on 14 Sept. 1960.

Initially, the animal weighed 7.53 g but at the time of death this had fallen to 2.33 g, less than one third of its original weight. From Fig. 2 it can be seen that the rate of water-loss declined steadily throughout the period of the experiment. On 17 Aug. the camel-spider was given a cockroach weighing 1.16 g, the net gain in weight after feeding being 0.42 g. It was allowed to drink its fill on 24 Aug., after which it showed a gain of 0.06 g, but still only weighed 3.89 g.

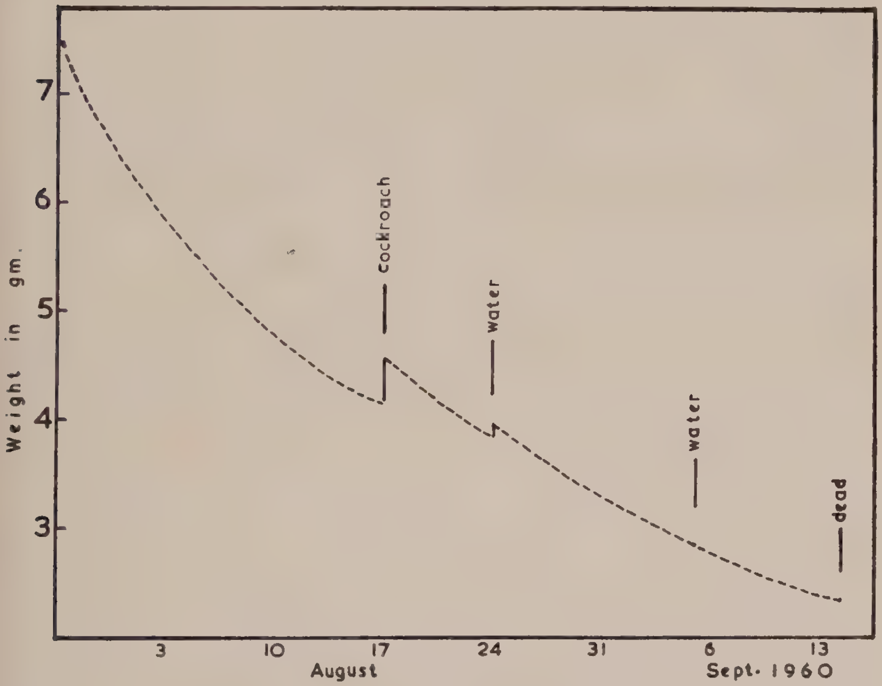


Fig. 2. Water-loss from a large female *Galeodes arabs* in dry air at room temperature 33° ± 2° C.

Comparable figures of water-loss have been obtained from other specimens, but those given above are adequate for discussion. A loss of 490 mg/72hr., the initial rate of water-loss from an animal weighing 7.53 g, represents a rate of water-loss of 0.116 mg/cm²/hr. if the surface area is calculated from the formula $S = k\, W^{2}/_{3}$, where S = surface area, W = weight and k is a constant. For a discussion of the significance of this method, see CLOUDSLEY-THOMPSON

(1959). In the present instance, a value of $k = 12$ was adopted, as with scorpions.

The figure of 0.116 mg/cm^2/hr. water-loss from *G. arabs* compares with 0.014 mg/cm^2/hr. at 19° ± 2° C from the fat-tailed scorpion, *Androctonus australis* (L.) of North Africa (CLOUDSLEY-THOMPSON, 1956) and 1.25 mg/cm^2/hr. at the same temperature from the West African species, *Pandinus imperator* (C. L. Koch) (CLOUDSLEY-THOMPSON, 1959). Although the room temperatures in London where the latter experiments were carried out averaged 14° C less than the room temperatures in Khartoum, the effect on transpiration was probably slight as both these temperatures are naturally well below the 'critical temperature' of the epicuticular wax-layer.

It is clear from this experiment, that camel-spiders show considerable powers of water-conservation compared with scorpions from wet tropical regions. On the other hand, they lose water some ten times faster than do desert species. This is probably correlated with the fact that they show a degree of speed and activity which far exceeds that of other Arachnida and may be associated with the development of a tracheal respiratory system in place of the lung-books characteristic of scorpions and spiders.

In hot weather, *G. arabs* will occasionally drink, but it does so clumsily. Specimens in captivity may lower their mouths into a watch-glass containing water, but seem to drink more easily from drops on a dead acacia leaf which they chew with their chelicerae. No doubt they seldom drink in nature, but obtain sufficient moisture from the body fluids of their prey.

AGGRESSIVE BEHAVIOUR

The reactions of *G. arabs* to stimuli such as the presence of other animals, sudden movements of nearby objects, vibrations of the substrate and other disturbances, consist of stereotyped patterns of activity which may be classified into the following categories:

Threat: The animal stands alert, swaying forward and backward, its pedipalps and first legs stretched forward and outward. At the same time chewing movements of the jaws are made.

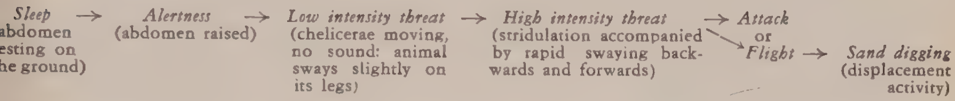
High intensity threat: Repetition of a mild stimulus or the introduction of a particularly disturbing one may result in a threatening response of higher intensity, which is usually accompanied by stridulation. This is produced by a more forceful and rapid movement of the jaws (whose inner surfaces bear stridulatory ridges) than occurs in the threat response of the first category.

Attack: The camel-spider turns towards the source of stimulation, rushes forwards and bites it. If the stimulus is caused by an object passing the animal while it is confined in a glass vivarium, attack results in an audible bang as the chelicerae strike the glass.

Flight: If a solifugid comes in contact with a source of intense stimulation, such as a scorpion or another camel-spider, it sometimes does not attack, but flees. In either case, contact is extremely rapid, lasting only a fraction of a second during which the solifugid appears to size up its adversary. This contact is made chiefly with the pedipalps which are the chief tactile organs.

Sand-digging: Much of the casual daily activity of Solifugae consists of sand-digging, but after extreme stimulation resulting in flight, an animal will often show an outburst of intense sand-digging.

The various states of activity of the camel-spider can be expressed quite simply as follows:



A resting solifugid in the state here described as 'sleep' is unresponsive to slight stimulations such as vibrations caused by a faint tap on the side of its cage. The abdomen and limbs rest upon the ground. Not infrequently the animal lies with the side of its abdomen resting on the sand.

Depending on the intensity of stimulation, the animal passes rapidly through the various states of alertness, low and high intensity threat. In the latter it stridulates, making a screeching sound which is caused by rubbing the inner ridged surfaces of the chelicerae against one another. It seems likely that stridulation has evolved from an "intention movement" (TINBERGEN, 1951), for many arachnids are known to clean their mouth-parts before beginning to feed (CLOUDSLEY-THOMPSON, 1958). A similar interpretation has been applied to stridulation in scorpions which may be regarded as elaborated from intention movements, or "displacement activities" associated with threat (ALEXANDER, 1958).

High intensity threat is followed by attack or flight, depending upon the physiological state of the animal. A camel-spider that has recently been fed is more likely to run away than a "hungry" individual and males are less aggressive than females. Thus males will usually flee from scorpions or a very active cockroach whilst females nearly always attack them. Sometimes a camel-spider will fly from the source of stimulation and begin sand-digging which may well represent a form of 'displacement activity' (CLOUDSLEY-THOMPSON, 1960; TINBERGEN, 1951).

FEEDINGS HABITS

The mode of feeding of Solifugae has often been described (BOLWIG, 1952; CLOUDSLEY-THOMPSON, 1958; HUTTON, 1843; TURNER, 1916, and others). The prey is apparently detected by touch and to some extent by sight and is then grabbed firmly by the chelicerae, sometimes assisted by the pedipalpal suckers. During feeding, the prey is moved sideways, to and fro through the camel-spider's jaws and macerated. The body contents are then sucked out to the accompaniment of perstaltic movements of the opisthosoma. Afterwards, camel-spiders can often be seen to clean their jaws in the sand.

In fights with scorpions and other Solifugae, the reactions of the camel-spider do not differ from their feeding responses. In most cases a male will escape from combat if this is possible, and in fights between the sexes the more aggressive female nearly always wins. Sometimes she gets a grip on the prosoma of her adversary between the chelicerae and almost severs them in a matter of seconds.

Whereas a hunting solifugid shows no hesitation over attacking prey smaller than itself, its behaviour when faced by larger animals is more hesitant and males will sometimes flee even from a vigorously struggling cockroach.

The response of a camel-spider depends both on the size and the activity of its prey. Males tend to bite and let go, females bite more quickly and are more tenacious, a policy that generally pays dividends in encounters with dangerous adversaries such as scorpions. On the other hand, the male's easily aroused flight reaction probably serves him in good stead wherever he comes across a female! Contrary to general opinion, camel-spiders are not only attracted to living prey; on occasion they will pick up a dead insect and begin eating it.

When disturbed in the act of feeding, camel-spiders usually adopt a threatening attitude, but do not readily attack or flee. Indeed, they are comparatively docile when their mouths are full and in these conditions can easily be manoeuvred from one cage to another. When feeding on a small insect, they will readily accept another and eat them both together.

RESPONSE TO ENEMIES

If a wandering scorpion should happen to touch a solifugid, both animals react instantly. The scorpion turns, with sting alert, to face its enemy, whilst the solifugid immediately flees in the opposite direction and may indulge in frantic digging. The camel-spider's instinctive "fear", to which this behaviour is attributed by HINGSTON (1925), is probably a response to the size and rapid movements of the scorpion. As its opponent lashes with its sting, the camel-spider leaps aside and if the two adversaries are constantly brought together, the same thing happens again and again. Especially if the solifugid is a male, however, the scorpion may succeed in delivering a fatal blow. The stricken camel-spider then staggers off and succumbs within three or four minutes. Female camel-spiders are more often successful than males. The scorpion's tail is grasped with lightening speed, and crunched between the jaws of its formidable enemy. It is then moved from side to side between the chelicerae of the solifugid and soon reduced to pulp.

Sometimes a camel-spider, in its initial attack, will grab a scorpion in its jaws and immediately begin feeding, more often than not first severing the sting of its foe (Fig. 3). This act does not appear to be due to any instinctive knowledge or intelligence on the part of the solifugid, but is probably merely a reaction to the most rapidly moving portion of the scorpion's anatomy. At other times, a camel-spider may attack again and again, pulling and tweaking the scorpion with its pedipalpal suckers until it can get a firm grip with its chelicerae and begin feeding.

Almost invariably, if a scorpion and a solifugid are separated after their initial encounter and then offered cockroaches, the scorpion at first seems unusually diffident, but the solifugid reacts to its new prey in a perfectly normal manner.

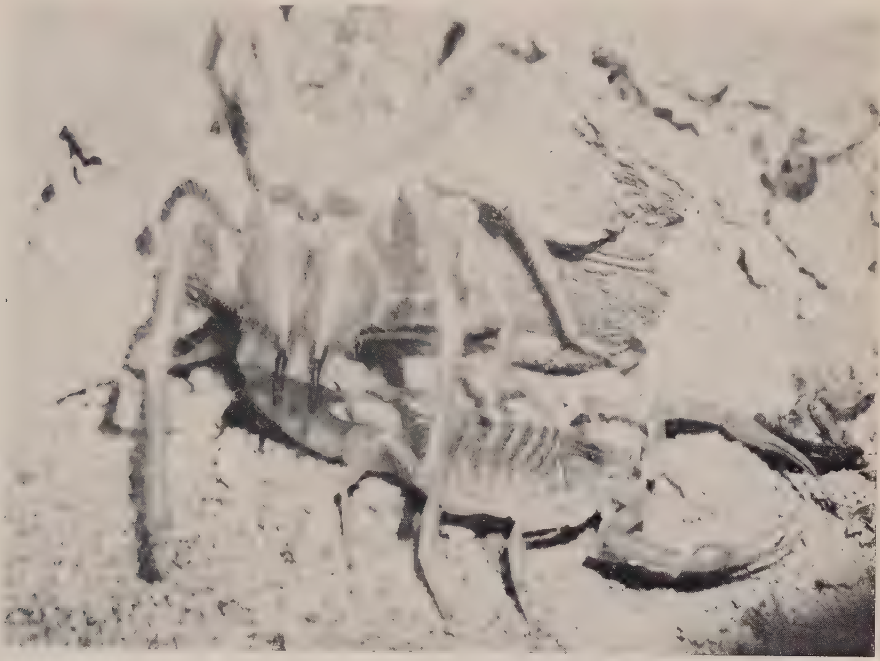


Fig. 3. *Galeodes arabs* attacking a scorpion, *Leiurus quinquestriatus* (H. & E.).

RESUME

QUELQUES ASPECTS DE LA PHYSIOLOGIE ET DU COMPORTEMENT DE GALEODES ARABS

Les moeurs nocturnes, particulièrement durant la saison chaude, des araignées *Galeodes arabs* ont été montrées par des expériences avec l'actographe.

Dans la nature elles passent la journée dans des abris profonds, dont l'ouverture est fermée par un tampon de feuilles mortes. Leur perte en eau par transpiration à l'air sec présente un taux assez bas et elles peuvent survivre à une forte dessiccation. Leurs réactions de comportement vis-à-vis des ennemis et des proies sont classées en promptitude, frayeur, frayeur très intense (accompagnée de stridulation) conduisant à l'attaque ou à la fuite (souvent suivie de déplacement avec rejet de sable). Les habitudes alimentaires sont décrites et on montre que la fuite se produit plus facilement chez mâles que chez les femelles.

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ÜBER DIE BILDUNG DER DIAPAUSEFORM BEI *TETRANYCHUS URTICAE* KOCH IN ABHÄNGIGKEIT VON GIFTRESISTENZ

VON

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Geprüft wurde die Bildung der Diapauseform bei Phosphorsäureester-anfälligen, retroresistenten, heteroresistenten und hochresistenten Stämmen von *Tetranychus urticae* Koch. Zu den bisher festgestellten biologischen Unterschieden zwischen anfälligen und resistenten Tieren wurde die Frage diskutiert, ob sie auf eine gemeinsame Ursache — das neurosekretorische System — zurückgeführt werden können.

Giftresistente Insekten- und Spinnmilbenstämme unterscheiden sich von ihren anfälligen Ausgangsstämmen vielfach in ihrer "Vitalität". So zeigten sich, wie in der Literatur weitgehend bekannt, bei *Musca domestica* L., *Blattella germanica* L. und *Tetranychus urticae* Koch mit der Resistenzsteigerung eine Verlängerung des Lebenszyklus, eine Verminderung der Nachkommenzahlen und eine verminderte Aliesterase (bei ersterer und letzterer).

In der vorliegenden Arbeit wird ein weiterer Unterschied zwischen anfälligen und resistenten Stämmen nachgewiesen: Die verschiedene Fähigkeit zur Bildung von Diapauseformen. Auf Grund dieser Ergebnisse wird versucht, die bisher bekannten Unterschiede unter einem einheitlichen Gesichtspunkt zu betrachten und damit zu einer Hypothese über den Mechanismus der Resistenz zu gelangen.

METHODIK

Drei Zuchten von *Tetranychus urticae* dienten dieser Arbeit: eine unbehandelte, gegen Phosphorsäureester (P) anfällige Rasse (LD_{50} = 0,00225% TEPP, HQ¹) = 4,3) aus dem Biologischen Institut der Farbenfabriken Bayer, die ursprünglich von Prof. Dosse, Hohenheim stammte; eine P-resistente (LD_{50} = 0,39% TEPP, HQ = 4,5) Rasse, die aus der oben genannten mit Systox ausgelesen wurde und eine heteroresistente ²) Rasse (LD_{50} = 0,013% TEPP, HQ = 269), die vor 2 Jahren resistent war und im Verlauf dieser Arbeit retroresistent (LD_{50} = 0,007%, HQ = 27) wurde. Abbildung 1 gibt die LD-Geraden dieser Stämme wieder.

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¹) Unter HQ (Heterogenitätsquotient) wird der Quotient $\frac{\text{LD95-Konz.}}{\text{LD50-Konz.}}$ verstanden der als anschauliches Maß für die Heterogenität einer Population dienen soll (MOERICKE & SABA, in litt.).

²) Als heteroresistent wurde ein nicht mehr behandelter Stamm bezeichnet, solange die LD50 ab- und seine Heterogenität zunimmt. Bei Abnahme der Heterogenität mit weiterem Rückgang der LD50 wurde der Stamm retroresistent (vgl. S. 265) genannt (SABA, 1961).

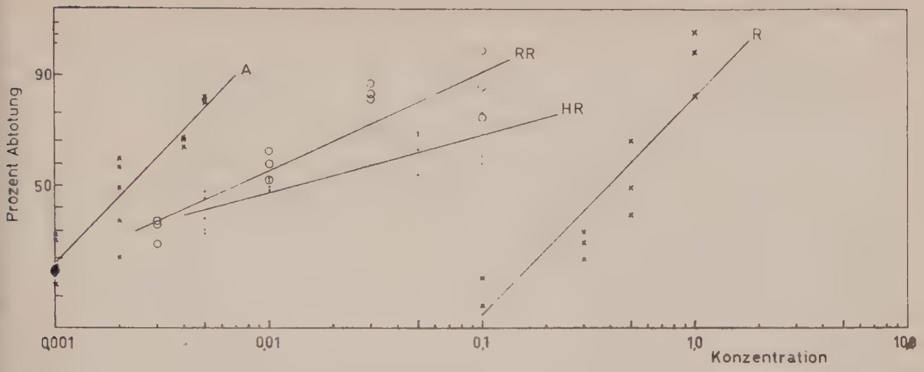

Abb. 1. LD-Geraden der in den Versuchen verwendeten Spinnmilbenstämme: A = anfällig, R = resistent, HR = heteroresistent, RR = retroresistent. Abszisse = LD50-Konzentration TEPP, Ordinate = Prozent Abtötung.

Diese Stämme wurden auf *Phaseolus vulgaris* unter Zimmerbedingungen mit 16h künstlicher Beleuchtung gehalten. Die Töpfe mit den Pflanzen standen innerhalb eines mit Perlongaze bespannten Kastens in Prilwasser, so daß die Stämme voneinander isoliert waren.

Bestimmung und Berechnung der Dosis lethalis (LD) wurden in einer früheren Arbeit beschrieben (SABA, 1961).

Die Versuche zur Bildung von Diapauseformen wurden in automatisch gesteuerten Klimaräumen unternommen, die mit künstlicher Beleuchtung (Osram-Leuchtstofflampen L40W/35,/15,/32 und /30) versehen waren.

BILDUNG DER DIAPAUSEFORMEN

Erkennung der Diapauseformen

Für die Diapause werden bei *Tetranychus urticae* bekanntlich besondere Winterweibchen ausgebildet. Es handelt sich um eine imaginale Diapause. Entscheidend für die Umstimmung sind Kurztag, tiefere Temperaturen und verschlechterte Nahrungsbedingungen (LEES, 1955). Wenn diese Faktoren während der jüngeren Entwicklungsstadien einwirken, entsteht im imaginalen Stadium die Diapauseform als Winter- (W-) Weibchen. Dieses saugt im Gegensatz zum Sommer- (S-) Weibchen nicht, bewegt sich wenig und ist anders gefärbt. Während jenes grünlich ist mit dunklen Flecken, nimmt das Winterweibchen Apfelsinen-E- bis Ringelblumenrot-E-Farbe an (vgl. Pflanzenfarben-Atlas, BIESALSKI, 1957). Weibchen, die eine Farbumstimmung zeigten, wurden auf frische grüne Bohnenblätter innerhalb eines Leimringes gesetzt. Dabei hat es sich herausgestellt, daß nicht alle, die einen solchen Farbwechsel aufwiesen — besonders die nur teilweise umgefärbten oder hell gefärbten —, echte Diapauseweibchen waren. Vielmehr saugten manche Tiere erneut weiter und erhielten nach einigen Stunden ihre Sommerfarbe wieder. Die echten Winterweibchen behielten demgegenüber eine ringelblumenrote Farbe, saugten nicht und bewegten sich wenig.

Bei den zu beschreibenden Versuchen wurde nur die echte Winterform berücksichtigt.

Diapause bei anfälligen und resistenten Weibchen

Beim Versuch 1 wurden je etwa 100 anfällige und heteroresistente S-Weibchen (vgl. Abb. 1 A und HR) gesammelt und auf jeweils 2 Käfige verteilt, die auf primäre Bohnenblätter aufgeklebt waren (DITTRICH, 1959). Hier konnten sie etwa 5 Stunden bei 26° C Eier ablegen.

Die abgelegten Eier wurden nach Entfernung der Weibchen gezählt und die Bohnen mit den Eiern in einem Klimaraum bei 13° C und 85% RL unter 9-Stunden-Licht gestellt. Nach 5 Wochen wurden die Käfige auf W-Weibchen, S-Weibchen und Männchen untersucht. Die Ergebnisse sind in Tabelle I zusammengestellt.

TABELLE I

Winterweibchen, Sommerweibchen und Männchen aus Eiern von Sommerweibchen eines anfälligen (A) und eines heteroresistenten (HR) Stammes bei 13° C, 9h Licht auf Bohne. 1 u. 2 = Wiederholung (August/September 1960)

Versuche	Eier	Imago	W.Weibchen	S.Weibchen	Männchen	⁰/₀ Diapause von Summe Weibchen	⁰/₀ Diapause von Summe Eier
A 1	115	68	36	12	20	*75,0*	31,3
A 2	95	58	27	10	21	*72,9*	28,4
Summe	210	126	63	22	41	*74,1*	30,0
HR 1	125	46	6	24	16	*20,0*	4,8
HR 2	140	68	6	40	22	*13,0*	4,3
Summe	265	114	12	64	38	*15,8*	4,5

Es ergaben sich eindeutige Unterschiede: Beim anfälligen Stamm entstand vorwiegend die Winterform (etwa 74%), beim heteroresistenten dagegen die Sommerform (etwa 84%). Diese beobachteten Unterschiede in der Bildung der Diapause zwischen den beiden Stämmen gaben Veranlassung zu weiteren Versuchen, um die Ergebnisse zu sichern.

Da der heteroresistente Stamm inzwischen anfälliger geworden war (vgl. Abb. 1, RR), sind in Versuch 2 (Tabelle II) zusätzlich Weibchen einer LD_{70}-Auslese dieses jetzt retroresistenten Stammes einbezogen worden. Hier wurden also S-Weibchen zur Eiablage aus den oben erwähnten 2 Stämmen sowie Weibchen, die eine LD_{70}-Auslese des RR-Stammes überlebten, verwendet. Es wurde ausschließlich mit der mehr langwelliges Licht enthaltenden L40W/32-Leuchte gearbeitet, um möglichst einen etwas höheren Prozentsatz an Diapauseformen zu erlangen. Im übrigen war die Durchführung dieses Versuches die gleiche wie in Versuch 1.

Im Versuch 3 wurde ein hochresistenter Stamm (Abb. 1, R) zu den anfälligen und retroresistenten Stämmen hinzugezogen und auf die Bildung von Diapauseformen geprüft. Die Bedingungen glichen denen des vorigen Versuches. Die Ergebnisse sind in Tabelle III zusammengestellt.

Auch die Ergebnisse dieses Versuches entsprechen denen der beiden vorigen; zwar gibt es Unterschiede zwischen den Wiederholungen der Gruppen, aber es bleibt deutlich erkennbar, daß die anfälligen mehr Diapauseformen bilden als die

TABELLE II

Winterweibchen, Sommerweibchen und Männchen aus Eiern von S-Weibchen eines anfälligen (A), eines retroresistenten (RR) und einer LD70-Auslese des retroresistenten Stammes von T. urticae *bei 13° C und 9h Licht. 1 u. 2 = Wiederholung. (November 1960).*

Versuche	Eier	Imago	W.Weibchen	S Weibchen	Männchen	% Diapause von	
						Summe Weibchen	Summe Eier
A 1	138	92	51	17	24	*75,0*	36,9
A 2	148	99	50	19	30	*72,5*	33,8
Summe	286	191	101	36	54	*73,7*	35,3
RR 1	230	128	30	50	48	*37,5*	13,0
RR 2	210	124	31	66	27	*32,0*	14,8
Summe	440	252	61	116	75	*34,5*	13,9
RR/LD70 1	105	42	8	27	7	*22,9*	7,6
RR/LD70 2	95	49	5	29	15	*14,7*	5,3
Summe	200	91	13	56	22	*18,8*	6,5

RR und HR (Versuch 1). Es ist anzunehmen, daß die entstandenen Diapauseformen bei den RR- und HR-Stämmen auf die anfälligen Tiere in diesen Populationen zurückzuführen sind. Bei dem hochresistenten Stamm betrug der Prozentsatz der Diapauseformen sogar nahezu null.

TABELLE III

W-Weibchen, S-Weibchen und Männchen aus Eiern von S-Weibchen eines anfälligen (A), eines retroresistenten (RR) und eines resistenten (R) Stammes von T. urticae *bei 13° C und 9h Licht. 1 u. 2 u. 3 u. 4 = Wiederholungen. (Januar 1961).*

Versuche	Eier	Imago	W-Weibchen	S-Weibchen	Männchen	% Diapause von	
						Summe Weibchen	Summe Eier
A 1	58	30	16	4	10	*80,8*	27,6
A 2	78	59	18	23	18	*43,9*	23,1
A 3	100	33	17	7	9	*70,8*	17,0
A 4	91	48	13	20	15	*39,4*	14,3
Summe	327	170	64	54	52	*54,2*	19,6
RR 1	55	38	8	17	13	*32,0*	14,5
RR 2	49	33	7	13	13	*35,0*	14,3
RR 3	90	32	3	17	12	*15,0*	3,3
RR 4	130	87	5	48	34	*9,4*	3,9
Summe	324	190	23	95	72	*19,5*	7,1
R 1	58	28	0	22	6	*0*	0
R 2	50	13	0	6	7	*0*	0
R 3	113	17	0	13	4	*0*	0
R 4	66	7	0	5	2	*0*	0
R 5	152	103	1	68	34	*1,5*	0,6
R 6	160	19	0	12	7	*0*	0
Summe	609	187	1	126	60	*0,8*	0,2

Um das zu verdeutlichen, wurden die Durchschnittswerte der Diapauseprozente (bezogen auf die Summe der Weibchen) bei den verschiedenen Stämmen als Funktion ihrer LD_{50}-Konzentrationen dargestellt (vgl. Abbildung 2). Die Abbildung zeigt deutlich, wie die Tendenz zur Bildung von Diapauseformen mit zunehmender Resistenz der Stämme abnimmt. Ferner geben die Ergebnisse Auskunft über die Mengenverhältnisse zwischen S-Weibchen, Männchen und Ge-

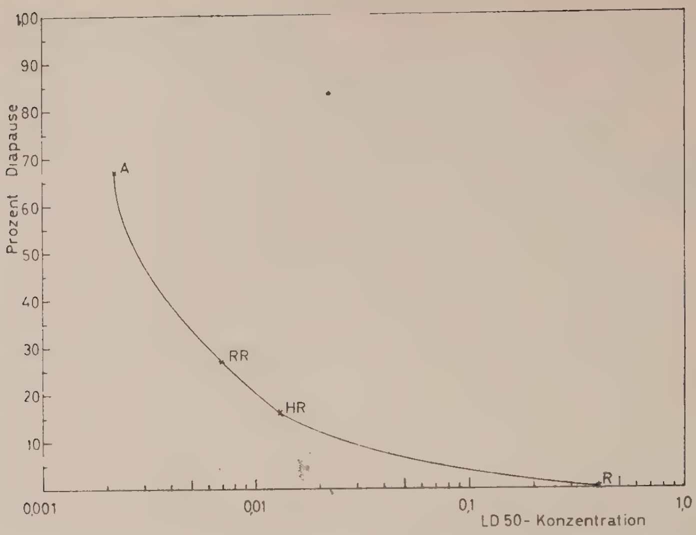

Abb. 2. Diapauseprozent bei einem anfälligen (A), retro- (RR), hetero- (HR) und hochresistenten (R) Stamm von *T. urticae* in Abhängigkeit von der LD50- Konzentration.

samtzahl der Imagines. Während der Prozentsatz der S-Weibchen bei höherer Resistenz zunahm (infolge der Abnahme der W-Weibchen), blieb der der Männchen etwa gleich (vgl. Tabelle IV).

Ausserdem ist zu erkennen, daß sich unter diesen Versuchsbedingungen nur ein Teil der abgelegten Eier bis zur Imago entwickelte. Die Durchschnittswerte zeigen, daß es sich um etwa 59% bei den anfälligen und retroresistenten Stämmen handelt; sie nahmen dann bei den höheren Resistenzgraden ab und betrugen bei den heteroresistenten 43% und bei den resistenten 31% der abgelegten Eier.

Die Vermutung von DITTRICH (1961), daß sich aus Eiern resistenter Weibchen ein höherer Prozentsatz an Männchen entwickelt als in einer normalen Population, konnte ich nicht bestätigen. Wie Tabelle IV aufweist, blieb das Verhältnis Weibchen zu Männchen bei allen Stämmen etwa gleich.

TABELLE IV

Durchschnittsprozente von Sommerweibchen, Winterweibchen, Männchen und Imagines bei den Versuchen zur Bildung von Diapauseform; Werte errechnet aus Tabelle I, II und III.

Stamm	% S-Weibchen bezogen auf Summe Imago	% W-Weibchen bezogen auf Summe Weib.	% Männchen bezogen auf Summe Imago	% Imago bezogen auf Summe Eier	Weibchen : Männchen
Anfällig (A)	23	67	30	59	2,3 : 1
Retroresistent (RR)	48	27	33	58	2,0 : 1
Heteroresistent (HR)	56	16	33	43	2,0 : 1
Hochresistent (R)	67	0	32	31	2.1 : 1

Die erhöhte Sterblichkeit während der postembryonalen Stadien bzw. der geringe Prozentsatz an Imagines bei dem R-Stamm im Vergleich zu dem A-Stamm ist deutlich (vgl. Tab. IV). Die Summe von Toten + S-Weibchen bei den R-Tieren (88%) ist fast gleich der von W-Weibchen + S-Weibchen + Toten bei den A-Tieren (84%), wie aus Tabelle III zu errechnen ist. Diese Feststellung deutet darauf hin, daß wahrscheinlich die R-Tiere, die sonst für die Diapause bestimmt gewesen wären, eingingen.

Diapause bei Männchen

Gasser (1951) berichtete, daß bei der Überwinterung vereinzelte Männchen beobachtet wurden. Ich konnte jedoch keine echten Diapausemännchen erhalten, wenn aus Eiern unbefruchteter anfälliger und resistenter Weibchen bei meinen Versuchsbedingungen (9h Licht, 13° C) Nachkommen heranwuchsen. Einige Männchen zeigten zwar eine geringe Farbumstimmung (siehe nächsten Abschnitt), konnten aber nicht eine Woche ohne Nahrung bei 6° C, 40% RL und 8-Stunden-Licht überleben. Es ist demnach anzunehmen, daß es Stämme von *Tetranychus urticae* gibt, bei denen die Männchen nicht zur Überwinterung befähigt sind. Wie später beschrieben wird, ist ihre Überwinterung wahrscheinlich auch nicht notwendig.

BEENDIGUNG DER DIAPAUSE

Die Winter-Weibchen wurden auf Bohnenblättern in Petrischalen in einen Kühlschrank mit eingebautem Licht gebracht und für 10 Wochen bei 6° C, 40% RL unter 8-Stunden-Licht (Osram L20W/32) gehalten. Danach wurden die überlebenden Weibchen (etwa $^1/_3$) einzeln bei 26° C auf frische, mit einem Leimring versehene Bohnenblätter gesetzt. Erst am nächsten Tag wurde Saugschaden beobachtet, und die Weibchen nahmen eine rostbraune Farbe an. Nach insgesamt etwa 40 Stunden begannen sie mit der Eiablage. Diese Zeit stimmt mit der überein, die Sommer-Weibchen von ihrem Schlüpfen aus dem Teleochrysalis-Stadium bis zum Beginn der Eiablage benötigen (eigene Ergebnisse). Eiablage und Weibchenfarbe entsprachen danach der Sommerform. Die aus diesen Eiern entwickelten Imagines ergaben sowohl Weibchen als auch Männchen. Folglich hat eine Begattung der Winterweibchen schon *vor* der „Überwinterung" im Kühlschrank stattgefunden. Daraus ersieht man, daß die Männchen möglicherweise nicht überwintern müssen.

DISKUSSION

Die Auslösung der Diapause der Insekten hängt mit der Hormontätigkeit zusammen. Lees (1955) sagt zusammenfassend über die *imaginalen* Diapausen:

> "Reproductive dormancy is probably controlled by the corpus allatum and perhaps, more remotely, by the brain. The relationship of the gonadotropic hormones with reproductive organs is direct; diapause supervenes if they are withheld."

Im Gegensatz zu den Insekten ist bei den Spinnmilben das Gehirn so eng mit den verschmolzenen Suboesophagal- und Abdominal-ganglienmassen verbunden, daß die Grenze zwischen diesen Organen nicht mehr klar zu erkennen ist (Blauvelt, 1945). Ob Corpora allata vorhanden sind, ist noch nicht untersucht. Es ist jedoch anzunehmen, daß auch bei Spinnmilben das Zentralnervensystem eine hormonale Steuerung bewirkt.

Die Ergebnisse dieser Arbeit zeigen, daß unter gleichen, für die Auslösung der Diapause günstigen Verhältnissen bei einem P-anfälligen Stamm von *T. urticae* 67% der Weibchen in Diapause eintreten, bei einem retroresistenten 27%, bei einem heteroresistenten 16% und bei einem hochresistenten Stamm nahezu 0%. Es bestände die Möglichkeit, daß das Optimum zur Auslösung von Diapauseformen bei R-Weibchen anders als bei den A-Weibchen liegt. Die Wahrscheinlichkeit hierfür ist aber nicht groß. Es ist eher anzunehmen, daß bei R-Weibchen keine Umstimmung zur Diapause erfolgt. Eine ähnliche Abhängigkeit vom Resistenzgrad zeigen auch verschiedene andere biologische Eigenschaften. So wurde eine verminderte Vitalität bei DDT-R-*Musca domestica* L. (MAELZER & KIRK, 1953; LICHTWARDT, WILBUR, DECKER & BRUCE, 1955; BARBESGAARD & KEIDING, 1955, u.a.), bei DDT-R-*Blattella germanica* L. (GRAYSON, 1953) und bei P-R-*Tetranychus urticae* (DITTRICH, 1961) festgestellt.

Verlängerte Larvalzeiten wurden beobachtet bei den 3 R-Stämmen der gleichen Tierarten im Vergleich zu den anfälligen (PIMENTEL, SCHWARDT & DEWEY, 1951; NORTON, 1953; GRAYSON, 1954; DITTRICH, 1961). Schließlich fanden sich ein erhöhter Lipoidgehalt bei DDT-R-*M. domestica* (REIFF, 1956; WIESMANN, 1957), eine verminderte Aliesterase bei P-R-Stubenfliege (VAN ASPEREN & OPPENOORTH, 1960) und bei P-R-Milben (VOSS, 1960) sowie stärker ausgebildete Fermentsysteme und Fermente, z.B. Cytochromoxydase, Phosphatase (SACKTOR, 1950, 1951).

Versucht man nun, die verschiedenen hier dargelegten Besonderheiten der giftresistenten Rassen auf einen Faktor zurückzuführen, so kann man vielleicht eine Änderung im neurosekretorischen System der Tiere annehmen. Dieses steuert ja ebenso die Diapause wie die Zahl der Nachkommen (WIGGLESWORTH, 1953), die Lipoid- und Fettmenge (NOVAK, 1959), den O_2-Verbrauch (DE WILDE & STEGWEE, 1958) sowie Fermentsysteme (NOVAK, 1959), Häutungen und Larvalzeiten (WIGGLESWORTH, 1953).

Da die Giftresistenz sich hauptsächlich gegen solche organischen Insektizide und Akarizide ausgebildet hat, die auf das Nervensystem einwirken, so könnte auch die Resistenz eine Beziehung zum neurosekretorischen System haben und der Resistenzmechanismus in Abhängigkeit von diesem System stehen.

Wenn die unter künstlichen Bedingungen gemachte Feststellung einer verminderten Fähigkeit zur Bildung von Diapauseformen bei R-Tieren auf Freilandverhältnisse übertragen werden kann, dann wäre zu erwarten, daß im Spätsommer und Herbst Winterweibchen nur als Nachkommenschaft mehr oder weniger anfälliger Weibchen entstehen. Demzufolge wäre eine Population in den ersten Generationen im Frühling anfälliger als im vorangegangenen Jahr vor der Überwinterung. Es wird vorgeschlagen, diese Folgerung durch Beobachtungen nachzuprüfen.

Herrn Professor Dr. H. Braun danke ich für die Arbeitsmöglichkeit und alle mir gewährte Hilfe. Herrn Professor Dr. V. Moericke bin ich für die kritische Beurteilung dieser Arbeit dankbar. Fräulein H. Schneiders bin ich für die Anfertigung der Photographien zu Dank verpflichtet.

SUMMARY

DIAPAUSE IN TETRANYCHUS URTICAE *KOCH IN RELATION TO RESISTANCE TO INSECTICIDES*

The production of the diapausing form of *Tetranychus urticae* was studied under constant conditions, with 9 hours light, 13° C and 85% relative humidity. Four strains with different degrees of resistance to organophosphorus insecticides were used. Their resistance to TEPP is shown in Figure 1 (page 265). One strain (A) was susceptible, another (R), very resistant; the others (RR = "retroresistent" and HR = "heteroresistent") were intermediate.

67% of the females of the susceptible strain entered diapause, none of the most resistant strain did so. The RR strain gave 27% and the HR 16% of diapausing individuals.

Some females showing apparently characteristic colour changes did not enter diapause. No diapause was seen in males.

The relationship between changes in the neurosecretory system in these different strains and the onset of diapause is discussed.

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EFFECTS OF VARIATIONS IN CASEIN AND DEXTRIN CONTENT OF A SYNTHETIC DIET ON LARVAE OF *CTENICERA AERIPENNIS DESTRUCTOR* (BROWN) (COLEOPTERA: ELATERIDAE)[1]

by

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Larvae reared aseptically on pellets of synthetic diet in which the ratio of casein to dextrin was varied over the full range, gained most weight in a two-month period when the ratio was either 20 : 80 or 70 : 30. When the ratio was varied by smaller amounts in the region of these concentrations, the larvae gained most weight in a narrow range of 21 to 23 parts casein and also in a broad range of 65 to 71 parts casein. This bimodal response may occur because deleterious effects of large quantities of casein or dextrin in the diet are counteracted by small quantities of the corresponding component.

A synthetic diet must satisfy the nutritional, physical and chemical requirements of an insect (FRIEND, 1955, 1958). The importance of the proper concentration of components in such diets for larvae of *Drosophila melanogaster* Meig. was demonstrated by improved and optimum growth when the protein content of the diet was raised to five per cent from the usual two per cent level (SANG, 1956). In the development of synthetic diets for wireworms, the effects of protein and carbohydrate levels appeared of initial importance. This is a report on the effects of varying the casein and dextrin concentration of synthetic diets on larvae of the prairie grain wireworm, *Ctenicera aeripennis destructor* (Brown).

MATERIALS AND METHODS

Groups of 25 larvae of *C. a. destructor* were reared individually and aseptically on each of 11 synthetic diets in which the casein and dextrin content varied by 10 parts (Table I). Similar groups of 25 larvae were reared on 12 additional diets in which the casein and dextrin content varied by 2 parts in the regions of 20 and 70 parts casein (Table II). These diets were supplied as pellets on the surface of moist absorbent cotton (DAVIS, 1959). The experimental period was two months. Those tubes in which fungal or bacterial contamination was observed were discontinued immediately and were not included in the final calculations.

The larvae were weighed to the nearest 0.1 mg at the beginning and at the end of the experiment. The larvae ranged in weight, initially, from 13 to 28 mg. The difference between the initial and final weights was recorded as milligrams change per milligram of initial weight.

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RESULTS AND DISCUSSION

Table I shows that different proportions of casein and dextrin did not affect the survival of *C. a. destructor.* When the fiducial interval was calculated at the 95% level, it was shown that larvae gained more weight when the diet contained 20 parts casein than with any other concentration except 30 or 70 parts casein. The peak occurring in the neighborhood of 20 parts casein and 80 parts dextrin was not unexpected, in view of the satisfactory growth and development obtained with other Coleoptera using these proportions (LEMONDE & BERNARD 1951, COOPER & FRAENKEL 1952, FRAENKEL 1955). The peak obtained with diets containing 70 parts casein and 30 parts dextrin was not anticipated.

TABLE I

Percentages surviving and average changes in weight in milligrams per milligram of initial weight when larvae of C. a. destructor *were reared individually and aseptically in test tubes on various synthetic diets provided as pellets on the surface of moist absorbent cotton for a period of two months.*

Parts Casein	Parts Dextrin	Survival		Change in Weight
		No.	Percentage	
0	100	20/21	95	0.44 ± 0.21*
10	90	17/18	94	0.80 ± 0.24
20	80	13/16	81	1.46 ± 0.25
30	70	15/16	94	0.96 ± 0.23
40	60	18/19	95	0.77 ± 0.24
50	50	15/20	75	0.73 ± 0.24
60	40	17/21	81	0.74 ± 0.24
70	30	15/20	75	1.18 ± 0.24
80	20	11/14	79	0.74 ± 0.25
90	10	16/18	89	0.60 ± 0.24
100	0	17/18	94	0.41 ± 0.24

* Standard Error.

Table II shows that as the casein content increased and the dextrin content decreased by two parts in the region of these two apparently optimal concentrations, maximum gain in weight was obtained at a level of 23 parts casein. This gain in weight at the 95% level of confidence was greater than that obtained with any other diet except those containing 21 or 67 parts casein. The significant gain in weight in the latter case was possibly due to the small sample size (Table II). At the 99% level of confidence, values obtained with 65, 67, 69 and 71 parts casein equalled those obtained with 21 and 23 parts casein. Although the difference was not sharp in the latter range, it was sufficient to substantiate the results obtained with larger increments (Table I).

If only small amounts both of casein and of dextrin are beneficial to the larvae, a concentration of 20 parts casein may be sufficient to overcome any deleterious effect of a large quantity (80 parts) of dextrin. A similar situation may exist at a dietary level of 70 parts casein, when a concentration of 30 parts dextrin may produce sufficient impetus to the larvae to overcome the harmful effect of so

TABLE II

Percentages surviving and average changes in weight in milligrams per milligram of initial weight when larvae of C. a. destructor *were reared individually and aseptically in test tubes on diets of low and of high casein content provided as pellets on the surface of moist absorbent cotton for a period of two months*

Parts Casein	Parts Dextrin	Survival		Change in Weight
		No.	Percentage	
15	85	15/19	79	0.54 ± 0.20*
17	83	11/17	65	0.69 ± 0.21
19	81	14/19	74	0.93 ± 0.21
21	79	14/18	78	1.24 ± 0.21
23	77	15/19	79	1.65 ± 0.20
25	75	13/18	72	0.82 ± 0.21
65	35	16/21	76	1.15 ± 0.21
67	33	6/10	60	1.22 ± 0.23
69	31	18/21	80	1.15 ± 0.21
71	29	19/22	86	1.16 ± 0.20
73	27	18/21	86	0.73 ± 0.20
75	25	16/22	70	0.85 ± 0.21

* Standard Error.

much casein. Between these concentrations, the interactions of the casein and the dextrin probably counter-balance each other.

The technical assistance of Mr. F. T. Eves is gratefully acknowledged.

SOMMAIRE

EFFETS DES VARIATIONS DU CONTENU EN CASÉINE ET DEXTRINE D'UNE ALIMENTATION SYNTHETIQUE DES LARVES DE CTENICERA AERIPENNIS DESTRUCTOR *(BROWN) (COLEOPTERA, ELATERIDAE)*

Des larves du taupin des graminées, *Ctenicera aeripennis destructor* (Brown), ont été élevées individuellement et aseptiquement sur des régimes synthétiques dans des éprouvettes pourvues de coton humide. D'une part, les régimes variaient de l'un à l'autre par une augmentation de 10 parties de caséine depuis 0 jusqu'à 100, pendant que la dextrine diminuait également par 10 parties depuis 100 jusqu'à 0. D'autre part, elles augmentaient de deux parties de caséine, à partir de 15 jusqu'à 25 parties et de 65 jusqu'à 75, pendant que la concentration de dextrine diminuait également de 85 jusqu'à 75 parties et de 35 jusqu'à 25 parties. Ces régimes étaient donnés sous forme de boulette sur le coton.

Les larves étaient pesées à 0.1 mg près au commencement des essais et la différence entre les poids initial et final de chacune était exprimée en quantités de milligrammes par milligramme de poids initial.

Les larves ont également bien survécu avec tous les régimes. La meilleure augmentation pondérale était obtenue avec un régime contenant 21, 23, ou 67 parties de caséine. Ce dernier résultat n'était pas du tout surprenant. Il est probable que ni un manque complet ni une surabondance de protéine ou d'hydrates de carbone ne profite aux taupins. Si les petites quantités peuvent favoriser l'accroissement des larves, il est fortement probable que 20 parties de caséine soient suffisantes pour détruire n'importe quel effet défavorable d'un surcroît de dextrine (80 parties). De même, une teneur de 30 parties de dextrine pourrait donner assez de vigeur aux larves pour surmonter l'effet d'un niveau alimentaire de 70 parties de caséine. Aux autres concentrations, l'action de l'un est antagoniste de l'autre et probablement leurs effets réciproques s'annulent.

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FORAGING BEHAVIOUR AND FEEDING HABITS IN FIVE SPECIES OF *TRINERVITERMES* IN WEST AFRICA

BY

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Foraging habits of five species of *Trinervitermes* occurring in W. Africa are described. The five species can be divided into two groups, comprising those which store grass fragments in the mounds and those which do not. Size distributions of stored grass fragments differ specifically. Experiments indicated that selective foraging of grass species may occur. Survival on various foods suggested adaptation to differing conditions in each species. The occurrence of all five species in one area seems to depend on a suitable mosaic of conditions.

This paper is one of a series of comparative studies of different aspects of the biology and ecology of five species of *Trinervitermes* (Isoptera, Termitidae, Nasutitermitinae) which occur in West Africa, viz.:

T. ebenerianus Sjöstedt
T. carbonarius Sjöstedt
T. oeconomus (Trägårdh)
T. auriterrae Sjöstedt
T. suspensus Silvestri

It has been suggested elsewhere (SANDS, in press) that the continued coexistence of five congeneric species could be explained in part by differences in foraging and mound building behaviour, the latter being described in detail. Distinct differences have also been found to occur in both foraging behaviour and the use of various food materials, and these are described in the present paper.

METHODS

The foraging habits of the five species were studied in two ways, by (1) Observations of foraging behaviour in the field, and (2) Measurements of samples of grass fragments obtained from the mounds, to find out whether size differences existed between species.

Two methods were also used to investigate the selection and utilisation of food materials, namely (3) Choice experiments and (4) Survival experiments. In the first of these, large samples of termites of all castes were given the opportunity to choose from a selection of common grasses in specially constructed glass chambers. These consisted of a glass box mounted across the middle of a rectangular plate, with access on two sides to flat foraging chambers. The grasses to be presented to the termites were passed through numbered perforations in paper strips so that they could be identified. The paper strips and grasses were clamped between glass strips and cemented in position with paraffin wax, after which the grasses were cut off to a standard length of 5 cm. One strip of grasses was placed across the end of each rectangular foraging chamber, and the amounts of each grass removed by the termites were measured at intervals of 24 hours (see Fig. 1).

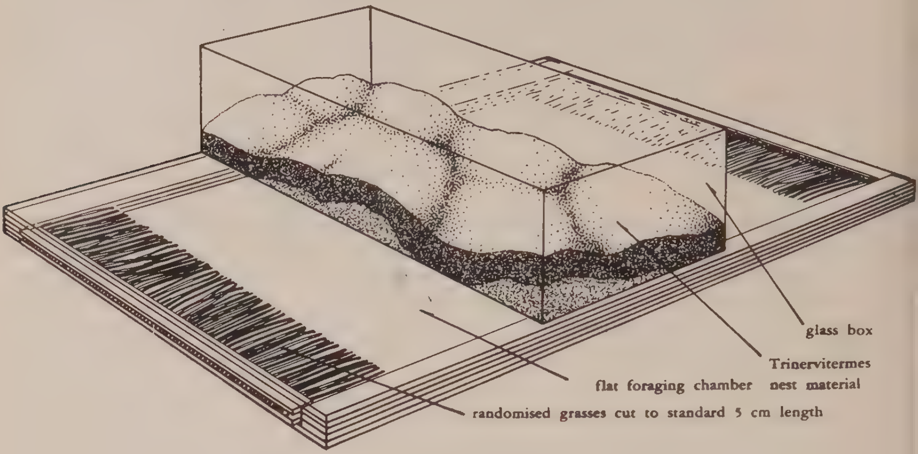
glass box
Trinervitermes nest material
flat foraging chamber
randomised grasses cut to standard 5 cm length

Fig. 1. Choice experiment set up in specially constructed glass chamber.

Six lengths of each grass were used, and 25 species were included in each experiment. The experimental layout was in effect a randomised block design, one replicate being used in each of six randomised lines of 25 species. Three lines in series made up the length of each paper strip exposed to attack in each of the foraging chambers.

The termites were extracted from portions of mounds brought into the laboratory, and were placed in the central box together with grass-free, moistened nest-fragments. These they quickly built into a complete cover, and generally commenced foraging within 24 hours. It was intended that the possible effects of non-random foraging arising from the establishment of fixed paths, particularly along the walls, and from interactions between favoured species and their immediate neighbours, should be minimised by the layout of the grasses. These experiments proved to be laborious in preparation, and the results were highly variable. They were therefore only used with the species well known to be vigorous foragers, namely *T. ebenerianus* and *T. carbonarius*.

The second type of experiment was similar in principle to those described in another paper in connection with *Odontotermes badius* (Hav.) (Sands 1956), based on survival of groups of worker castes only, supplied with different food materials. These experiments were restricted to workers because the other castes, being dependant on the workers, would introduce an extra uncontrolled factor. The termites were confined on a layer of sterile moistened sand in 9 cm petri dishes with the food material under test. The dishes were kept in a wide tank over standing water during the experiment, to maintain high humidity. No steps were taken to maintain a uniform temperature. Five replications, each of fifty termites, were used to test each food material. The insects were handled with soft brushes throughout, but all those dying within the first 24 hours were regarded as handling injuries, and were replaced by live individuals. It is estimated that by this means handling injuries were reduced to 0.05% of the total mortality. The dead termites were recorded each day thereafter and removed to prevent their being eaten by the remainder.

RESULTS

Observations on foraging behaviour

According to GRASSE & NOIROT (1951), "The *Trinervitermes* of Africa carry out foraging excursions almost every night, in the course of which they gather debris of grass, seeds, etcetera. The columns proceed in the open air, and the individuals of which they are composed make a practice of marking their trail with their excrement".

Observations on the five species named indicate that this statement is not universally applicable, since they can be divided into two groups, comprising those which have the "harvesting" habit and store grass fragments in their nests, and those which do not. The first group includes *T. ebenerianus, T. carbonarius,* and *T. suspensus*, and the second, *T. oeconomus* and *T. auriterrae.* Extensive digging in the mounds of the two latter species at various times of the year has never revealed more than a very few dusty fragments, and usually none at all. It may also be noted that in the first three named, the pigmented parts of both soldiers and workers are considerably darker in colour, being dark brown or even black, whereas in *T. oeconomus* and *T. auriterrae*, the workers are light brown and the soldiers yellow or yellow-brown with darker nasus. That the colour grouping follows that of the habits may not be without significance.

The dissection of foraging workers has revealed that the crop in most cases contains quantities of the material being worked. Fragments of fresh green grass were found in the oesophagus and crop of *T. ebenerianus* workers. The workers of all five species thus appear to feed whilst foraging, and the main difference between the two groups lies in the behaviour of workers returning to the mound. In the first group, a considerable proportion carry or drag fragments of grass or other food material with them. In the second, such particles as are carried back usually only fill the space between the mouthparts, and are presumably consumed soon after arrival. Specific differences in foraging behaviour were also observed among the five species. In *T. ebenerianus*, the commonest and most vigorous forager of the northern *Isoberlinia* savannah-woodland (AUBREVILLE, DUVIGNEAUD, HOYLE, KEAY, MENDONÇA & PICHI-SERMOLLI, 1959), vegetation type 17) most of the foraging took place at night, but in mild dull weather would sometimes continue after daybreak. Foragers emerged either from small holes in the mound surface or more commonly from subterranean tunnels at a distance of several yards from the mound. Soldiers accompanied the workers, and also lined the route at intervals of about 1 cm, their heads pointing outwards; minor soldiers were in the large majority. Large parties often spread out over an area of 25—30 square feet, and workers frequently climbed the tall savannah grasses to a height of 5—6 feet. Fragments of leaves and inflorescences cut off at this height were carried or dropped to the ground, in the latter case usually accompanied by the termite. The degree of abundance and activity of this species is indicated by the fact that at night a large group of foragers was often audible for several yards before it was located by sight.

T. carbonarius was never observed to forage as extensively or vigorously as *T. ebenerianus*. Foragers sometimes emerged from small holes in or near the mound, but if any distance was covered, mud runways were constructed over the surface of the soil, often for distances of several yards. The quantities of grass fragments stored in the mounds were not as great in this species as in *T. ebenerianus* even at the peak foraging periods of the year.

The smaller species, *T. suspensus*, had similar foraging behaviour to that of *T. ebenerianus* which it closely resembled apart from its size. It collected mainly the smaller finer species of grass, and stored considerable amounts, though not as much in proportion as its larger congener.

In the "harvesting" species in Northern Nigeria, foraging was found to be a seasonal activity, being discontinued during the wettest months of the year, that is, July, August, and early September, and also during most of the cold dry weather of November to February. In these months, certain nights presented less adverse conditions, and sporadic foraging would take place. It became active again when increasing temperature and humidity heralded the beginning of the rainy season, in March and April, and continued during the early storms of May and June. Late September, and all of October were also months in which foraging activity was intense.

The foraging behaviour of *T. oeconomus* was distinctly different from the above. The mound has been described in another paper (SANDS, in press) as having permanently open holes in its surface. From these and from holes in the ground nearby, the foraging termites spread among the vegetation near the mounds. Long columns were not observed in this species, and the termites browsed for a longer period. There appeared to be less movement to and from the mound. The mounds of this species were often situated in sheltered positions under trees, and on a number of occasions the termites were found working beneath dead leaves and other vegetation. Late worker and alate nymphs were sometimes found accompanying these foragers, which continued their activities during the earlier part of the day, and also during less favourable weather conditions when foraging by the "harvesting" species had ceased.

The behaviour of *T. auriterrae* appears to be similar to that of *T. oeconomus*, though because of its scarcity it could not be observed as frequently. One short foraging column was seen, travelling a few feet from a hole in the soil surface to a patch of dead grass. This species has also been found working in and under piles of dead vegetation including grass, sheltered by trees, during the daytime. It is the only *Trinervitermes* species in this region which was found building mud runways up trees, though this has also been recorded in an Indian species, *T. biformis* Holmgren (PATEL & PATEL, 1954). It has been collected from under dead wood on which it had been feeding, and has even been recorded as causing slight damage to young growing trees.

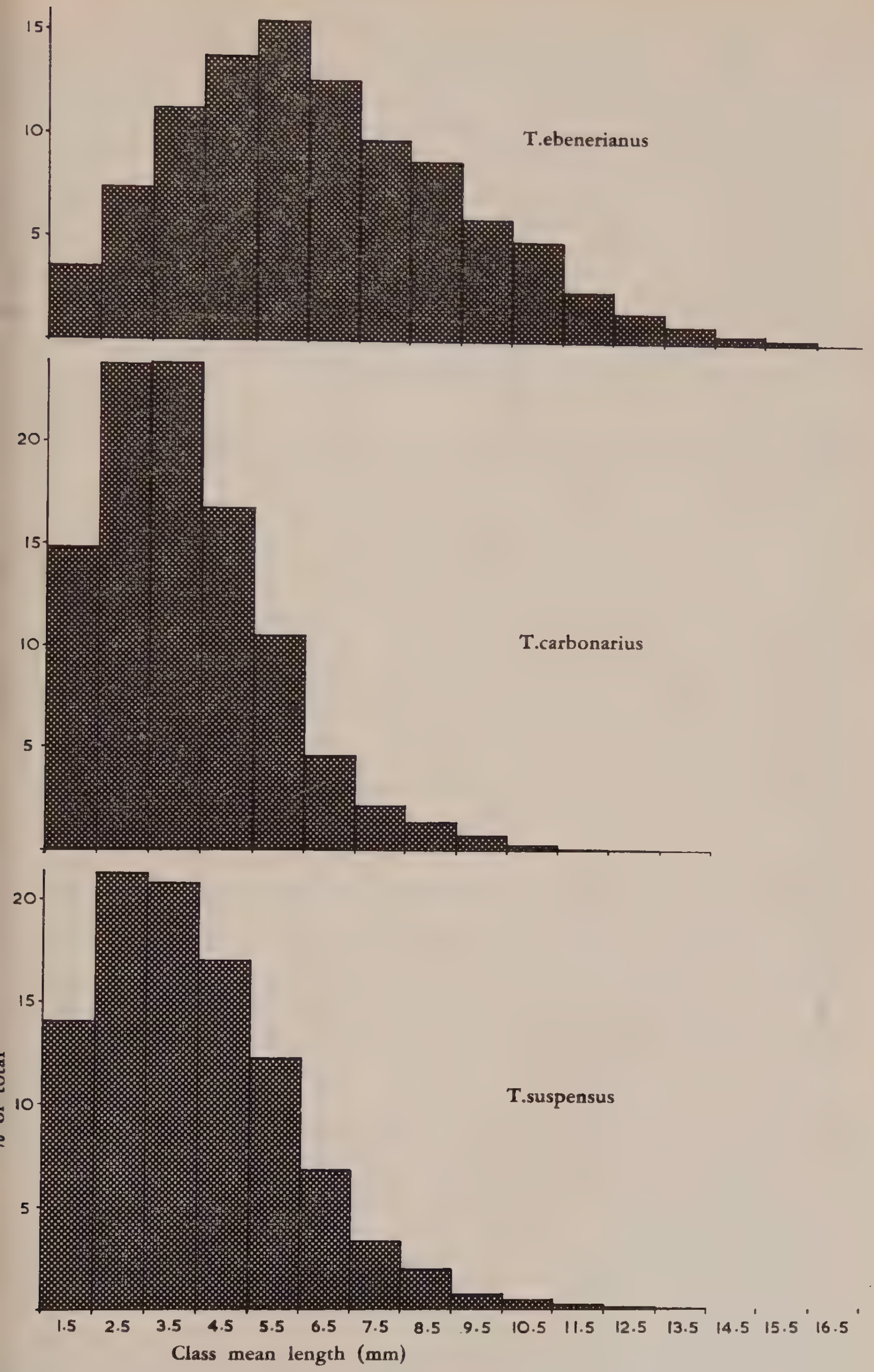

Fig. 2. Comparison of size distribution in samples of stored grass cuttings from the mounds of three species of *Trinervitermes*.

Measurements of grass fragments from the mounds

Large samples of grass fragments from five mounds of each of the three "harvesting" species were measured. Histograms have been constructed of the percentages of each size, to enable comparisons between them to be made (Fig. 2). It was found that *T. ebenerianus* carried back to the mound and stored larger fragments than the other two species, the mode of the distribution being at the 5—6 mm size group, and the mean length 6.26 mm. *T. carbonarius* has workers as large as those of *T. ebenerianus*, but the mode was in this case at 2—4 mm, the mean length being only 3.76 mm, which was smaller than in *T. suspensus*. The latter stored fragments of mean length 3.97 mm, the mode of the distribution being similar to *T. carbonarius*, but a slightly greater proportion of longer fragments occurred.

TABLE I

Results of choice experiments with T. ebenerianus, *expressed in terms of mean percentage of each grass removed. Where a dash (—) is shown, the grass species was not included in the experiment, nil attack being shown by O.*

Grass species	Expt. I all parts of grass	Expt. II stems only	Expt. III leaves only	Expt. IV leaf in 0.2 cm strips
Eragrostis sp.	—	76	81	96
Brachiaria jubata (Fig. et De Not.)	72	84	13	76
Hackelochloa granularis (L.)	59	—	—	—
Paspalum scrobiculatum L.	71	100	29	29
Rottboellia exaltata L.	43	97	19	66
Eragrostis sp.	—	29	74	66
Andropogon sp. (small, possibly *calvescens* Stapf)	45	—	—	—
Pennisetum polystachyon (L.)	35	70	8	62
Beckeropsis uniseta (Nees)	—	60	34	39
Schizachyrium exile (Hochst.)	46	24	30	70
Monocymbium ceresiforme (Nees)	57	10	39	59
Panicum sp. (not *P. maximum*)	75	21	3	62
Pennisetum purpureum Schum.	64	49	10	37
Andropogon pseudapricus Stapf	59	31	6	61
Setaria sphacelata (Schum.)	25	97	11	23
Pennisetum pedicellatum Trin.	45	38	22	38
Urelytrum muricatum C. E. Hubbard	15	17	57	49
Loudetia simplex (Hack)	48	4	10	66
Andropogon gayanus Kunth.	30	18	0	81
Elionurus tenax Stapf	31	—	—	—
Hyparrhenia subplumosa Stapf	30	—	—	—
Schizachyrium brevifolium (Schwartz)	61	22	3	—
Ctenium elegans Kunth.	54	10	6	43
Loudetia togoensis (Pilger)	26	—	—	—
Hyparrhenia rufa (Nees)	—	9	5	60
Rhytachne triaristata (Stend.)	22	—	—	—
Hyparrhenia cyanescens (Stapf)	—	38	1	24
Andropogon schirensis Hochst ex A. Riches	3	16	22	36
Aristida kerstingii Pilger	29	6	0	0
Cymbopogon giganteum (Hochst.)	—	0	4	22
Size of significant difference between means, i.e., $2 \times t_{0.05}\, s/_{\bar{x}}$	—	52	44	70

TABLE II

Results of choice experiment with T. carbonarius. *The two columns show the earlier preferences and the wider range consumed when the preferred grasses were nearly finished*

Grass species	Leaf in 0.2 cm strips	
	Results after 2 days	Results after 4 days
Eragrostis sp.	99	100
Rotboellia exaltata	93	97
Brachiaria jubata	92	97
Andropogon gayanus	87	95
Pennisetum purpureum	67	94
Urelytrum muricatum	65	94
Panicum sp. (not *P. maximum*)	65	87
Pennisetum polystachyon	60	90
Hyparrhenia rufa	54	91
Setaria sphacelata	53	86
Pennisetum pedicellatum	51	84
Andropogon pseudapricus	47	86
Loudetia simplex	47	86
Hyparrhenia cyanescens	43	91
Eragrostis sp.	31	79
Beckeropsis uniseta	30	75
Schizachyrium exile	29	73
Monocymbium ceresiforme	21	84
Paspalum scrobiculatum	20	89
Ctenium elegans	19	41
Cymbopogon giganteum	15	72
Andropogon schirensis	12	77
Schizachyrium brevifolium	4	24
Aristida kerstingii	0	0
Size of significant difference between means, i.e., $2 \times t_{0.05}\, s/_{\bar{x}}$	38	—

Choice experiments

In early experiments in the choice chambers, the grasses used were of various thicknesses with the result that larger quantities of the thicker grasses had to be removed to score the same length as smaller ones. This was avoided in later experiments by cutting strips of grass to as nearly constant width as possible. The results are expressed in terms of mean percentage of each grass removed in Tables I (*T. ebenerianus*) and II (*T. carbonarius*). Analyses of variance were carried out on the results of each of the four later experiments. The differences required for significance to be attained are given at the foot of the columns in each table. They show considerable variation between different experiments, arising from the variable behaviour of the termites, in that a sample from one colony would forage more or less at random, and others established regular pathways. It was found that the position of the grass produced a significant effect due to non-random foraging in three of the four experiments analysed. Significant differences occurred between the more widely separated mean percentages removed in each of these.

It would appear from the results that a few grasses are consistently avoided by both species of *Trinervitermes* if there is any alternative. Most would seem to be more or less palatable, and the variation which occurred between colonies suggested that familiarity with a group of grasses which are normally available near the mound might be an important factor governing the selections made. Some grasses accepted by one colony were avoided by another. The final results of each experiment are shown in the table in the case of *T. ebenerianus*, but in the single experiment with *T. carbonarius* the two sets of figures show the earlier preferences and the wider range consumed when the preferred grasses were nearly finished. In the later case analysis was not undertaken because too many replicates had been completely removed. The grasses are arranged in order of preference in both tables, but in Table I it is the average preference over the four experiments. The highest in that order are mainly the smaller finer grasses, which are comparatively scarce in the savannah, with the larger, commoner species forming the middle part of the list. The two least eaten were *Aristida kerstingii*, which is a tough resistant grass common on very poor dry soils, and *Cymbopogon giganteum*, which is a member of an aromatic genus. *Cymbopogon citratus*, "Lemon grass", was not tested, but is reputed by the local Africans to repel insects, including termites (RAINS 1937). *C. giganteum* has a similar though less pronounced aromatic content.

The list of grasses tested is by no means exhaustive, and the experiments are to be regarded only as indicating that discriminative foraging is likely to occur where possible. HARTWIG (1955) stated that the S. African species of *Trinervitermes* carried away the grasses in the immediate vicinity of the nest in the same proportions as they occurred within a thirty-foot radius of the mound. These grasses included a species of *Aristida, A. congesta*, which in one case composed 90% of the stored grass. This was however on poorly covered veld where the termites were estimated to remove from 20—60% of the grass cover, and discriminative foraging may have been impossible owing to shortage of preferred species.

Survival experiments

The possible food materials tested by means of the survival of groups of worker termites consisted of the following: freshly cut live green grass; dry dead grass, from the termites' own mound as far as possible; rotting leaves (of trees or shrubs known to be consumed by other termites); rotting wood, with the same proviso; pure cellulose in the form of filter paper cut into grass-sized strips. Starvation was used as a control treatment, and dry grass was regarded as a control at the other extreme. The tests were carried out in two parts: first the "natural" foodstuffs were tested against starvation, and then pure cellulose was tested against dead grass and starvation. It was also found necessary to separate the species into two groups. *T. ebenerianus, T. carbonarius*, and *T. oeconomus* were easily obtained in sufficient numbers and were tested together. *T. suspensus*, though common, was seldom found in sufficient numbers, and *T. auriterrae* was comparatively rare. To obtain adequate supplies of these two species required longer searching, and they were therefore tested together in later experiments.

The results of these experiments were essentially similar to the "time/mortality" data arising from certain types of bioassay of insecticides, such as were described by CALLAWAY, DIRNHUBER & WILSON (1952) and WHEATLEY & HARDMAN

(1960, 1960a). These authors related the dose of insecticide to the time taken for 50% of the test animals to respond (by death). In the present case, the times required for the termites to reach 50% mortality were taken to indicate the values of the different food materials tested. The results were however complicated by an unknown "natural" mortality, particularly where the available food promoted longer survival, owing to age differences in the samples of termites taken at random from their mounds.

In view of the wide variation which occurred between replications of the same treatment, it was considered preferable to calculate the 50% mortality-times for each replication of each treatment individually, and to make these the basis for analysis of variance on treatments and replications, rather than to combine the five replications and calculate a single 50% mortality-time for each treatment. A sample of the 50% mortality-times was obtained graphically by plotting percentage mortality on the probability scale against time in days on a logarithmic scale, this relationship being linear over most of the percentage range. The figures so obtained differed only slightly from the 50% mortality-times calculated arithmetically from the primary data. These differences were insignificant compared with the variation between replications, and in any case tended towards a constant proportion of the 50% mortality-times. The arithmetically calculated 50% mortality-times were therefore used in the analysis of variance.

TABLE III

Survival of 5 species of Trinervitermes *on different possible food materials. Figures are mean 50% response times of 5 replications of each treatment expressed in days.*

Species	Treatments					
	fresh green grass	dry dead grass	rotting leaves	starvation	rotting wood	Sign. diff. = $2\,(t_{0.05}\,s/_{\bar{x}})$
T ebenerianus	9.74	23.70	21.88	14.64	21.10	8.64
T. carbonarius	21.04	25.00	18.36	10.72	13.12	7.45
T. suspensus	2.56	6.52	10.88	2.40	9.82	3.88
T. oeconomus	10.00	20.46	17.96	9.42	11.42	8.02
T. auriterrae	9.40	24.41	20.14	3.18	14.06	5.21

The results for all five species of *Trinervitermes* are shown in Tables III and IV. The figures given are the mean 50% mortality times for each treatment, and the significant differences are twice the fiducial semi-intervals at 5% probability. Table III shows the results for the "natural" foods, and Table IV the comparison of pure cellulose with starvation and dry grass controls.

In three of the five species, survival on fresh, green grass clippings (from a species of *Eragrostis* high in the order of preference in the choice experiments) was not significantly different from starvation, and in *T. ebenerianus* there was even a tendency towards a detrimental effect. In view of the presence of fresh green grass in the crops of foragers of this species, this is hard to account for, but it may be that only small quantities are consumed immediately in the natural state, the rest being stored until dried in the mounds. In *T. carbonarius*, fresh green grass promoted survival not significantly different from dead grass, which

TABLE IV

Utilisation of cellulose by 5 species of Trinervitermes. *Figures are as in Table III.*

Species	Treatments starvation	 dry dead grass	 pure cellulose	Significant difference $= 2\,(t_{0.05}\, s/\bar{x})$
T. ebenerianus	21.26	37.00	27.80	5.98
T. carbonarius	21.12 (25.42)	42.20 (50.10)	41.74	27.15 (17.00)
T. suspensus	5.10	8.92	3.66	2.87
T. oeconomus	17.20	36.20	16.78	9.27
T. auriterrae	6.00	8.44	5.70	2.12

in most cases appeared to be the optimum diet, and in *T. auriterrae* green grass was better than starvation, though not as good as other diets. The survival of *T. ebenerianus* on rotting leaves and rotting wood, though not significantly different from starvation, should probably be regarded as grouped with dry grass, to which they are much closer. The marginal differences in *T. suspensus* between dead grass, and rotting leaves and wood, may be significant. In the tests of cellulose, the results suggest that the ability to make use of cellulose in a pure state may have been retained to some extent in *T. ebenerianus*. In *T. carbonarius* the results were rendered non-significant by two aberrant replications, one of which occurred in starvation, and the other in the dead dry grass treatment. They showed abnormally high mortalities, presumably owing to disease or other unidentifiable factors. If these two are treated as missing values, the re-calculated analysis of variance shows pure cellulose to be very nearly significantly better than starvation. The re-calculated figures are given in brackets in Table IV. The results of the other species showed no difference to occur between starvation and pure cellulose.

DISCUSSION

The results described above have enabled some assessment to be made of the ecological status of the five species of *Trinervitermes* which were found in the same habitat.

In *T. ebenerianus*, the "harvesting" habit is most strongly developed. This was indicated by its very intensive foraging activity during which more and larger grass fragments were collected and stored than in any of the other species. Much of this activity took place at times when the grasses were dry or at least mature, and this may account for the apparently adverse effect of fresh young green grass as a sole source of food. So far as dead plant material was concerned, this species appeared to be adaptable, though surviving best on its own choice of food. It has been observed to collect small quantities of other materials, including caterpillar grass, whilst foraging. It is considered that *T. carbonarius* represents a lower stage in the development of the "harvesting" habit. Its survival response to fresh green grass suggests an adaptation to more moist conditions than *T. ebenerianus*, and its largest colonies were in fact observed in the southern, moister savannah and forest-savannah mosaic areas (Keay et al., 1959), vegetation types 16 and 8 respectively). The more strongly developed habit of constructing covered earth runways may also be an adaptation to foraging under conditions of more frequent rainfall, as a means of protection to the termites from being dispersed by sudden rainstorms. In the northern *Isoberlinia* savannah-woodlands, *T. carbonarius* reaches the northernmost limit of its geographical range, and it was not found in the drier "Sudan" savannah-woodland (KEAY et al., type 20).

T. suspensus appeared to be intermediate between *T. carbonarius* and *T. ebenerianus* in the possession of a habit of grass storage, but it has diverged from them in becoming very largely dependant on the existing mound structures of other species. Though closely related morphologically to *T. ebenerianus*, and common, it would thus appear to have ecological requirements which are unlikely to compete with its larger congeners.

Of the two species which do not store grasses, *T. oeconomus* appeared the less adaptable in the matter of survival on different foods. Rotting leaves and dry grass were of equal value, agreeing with the location of its mounds and foraging, which continued in these sheltered situations almost throughout the year. The lack of any grass storage habit in this species, and the consequent need for continuous foraging, may have given rise to its more frequent occurrence in sheltered situations.

T. auriterrae similarly is most often found in sheltered habitats, but appeared more adaptable in retaining some ability to make use of woody material. It also shares to some extent the adaptation to moister habitats shown by *T. carbonarius*, in its ability to survive for a while on green grass alone and in the construction of earth runways. Though less common than *T. carbonarius* it has a wider geographical range, having been found in the "Sudan" savannah-woodland. It is thought that *T. auriterrae* may be the nearest of the group of species studied to a non-"harvesting" *Nasutitermes*-like ancestor, on the grounds not only of its foraging and feeding habits, but also on the internal structure of the mound. The faecal "carton" lining of the galleries has on its surface a well developed shagreened pattern similar to that which occurs in some *Nasutitermes* species. In the other four *Trinervitermes* species studied this pattern is vestigial.

Thus in these few species of the genus is perhaps indicated the probable course of adaptation, from the wood-feeding *Nasutitermes*-like ancestor, confined to forests or well wooded habitats, to the highly successful open savannah forms.

The occurrence of all five species in a single large-scale habitat in West Africa, such as the northern *Isoberlinia* savannah-woodland, would seem to depend on the occurrence of a suitable mosaic of conditions on a smaller scale. This results in segregation of the species in a manner parallel to that described in ants in a temperate habitat by BRIAN (1956).

RESUME

COMPORTEMENT D'APPROVISIONNEMENT ET MOEURS ALIMENTAIRES CHEZ CINQ ESPÈCES DE TRINERVITERMES *EN AFRIQUE OCCIDENTALE*

Ce travail est l'un d'une série d'études comparatives de biologie et d'écologie de cinq espèces de *Trinervitermes* en Afrique Occidentale. On y décrit les habitudes d'approvisionnement et les différences spécifiques. Les cinq espèces forment deux groupes, contenant

l'un, *T. ebenerianus*, *T. carbonarius* et *T. suspensus*, lesquels entassent dans leur nids des fragments d'herbe; l'autre, *T. oeconomus* et *T. auriterrae*, lesquels n'en entassent pas. Les fragments d'herbe entassés sont de taille différente selon les trois espèces. Les expériences sur les deux espèces *T. ebenerianus* et *T. carbonarius* suggèrent que des approvisionnements sélectionnés d'espèces d'herbe peuvent se produire quand c'est possible. L'adaptation aux différentes conditions pour chaque espèce est suggerée par la survie des ouvriers qui se nourrissent différemment. On pense que *T. auriterrae* se rapproche le plus d'un ancêtre qui ressemble à *Nasutitermes*. La coexistence des cinq espèces dans un même habitat étendu, paraîtrait dépendre de la présence d'une mosaïque de conditions appropriées sur une plus petite échelle

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FIELD STUDIES OF THE DAILY ACTIVITY AND FEEDING BEHAVIOUR OF SUNN PEST, *EURYGASTER INTEGRICEPS* PUT., (HEMIPTERA, SCUTELLERIDAE) ON WHEAT IN NORTH IRAN

BY

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The horizontal and vertical movements and the daily feeding activity of six young adult Sunn Pest, each tagged with a small piece of Ta^{182} so that it could be located, were studied in wheat fields in north Iran for 3 to 4 days at the start and at the end of the adults' feeding period in June 1960. The results are described and discussed.

The Sunn Pest, called "Senn" in Iran, (*Eurygaster integriceps* Put.) is a serious pest of wheat, and to a lesser extent barley, in the Middle East and countries bordering the Caspian Sea. The insect is well known for its migratory habits and for the well defined annual cycle of changes in its physiological state in adult life. Having first reached the adult stage in the wheat fields in early summer, it feeds intensively on the wheat ears for several days before migrating (often over long distances) to mountains where it hibernates.

The insect's behaviour has often been observed but descriptions are sometimes conflicting and are rarely supported by evidence. One of the objects of the work described below was to produce evidence of the behaviour of the young adult at the beginning and end of the feeding period in the wheat field, and in wheat crops where environmental conditions differed.

Information on the insect's behaviour is necessary for reliable estimates of the density of the pest on wheat, because the extent of horizontal and vertical movements and their relation to time of day and to microclimatic conditions in the wheat crop has to be taken into account when assessing the value of different methods of sampling; in fact, the work was done partly as a basis for a comparative study of methods of estimating density by sampling of the young adult insects during the feeding period in mid-summer in northern Iran. Information

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on the extent of horizontal movements is especially important when estimating density by counting the insects found in randomly-selected quadrats of 1 square meter and by marking-release-recapture methods. Information on vertical movements at different times of day is important not only for sampling by sweep-net but also for control by spraying with contact insecticides.

Several young adult *Eurygaster integriceps* were observed closely and frequently for several days at a wheat field near Mamazan, a village in north Iran, during June 1960. To locate the insects during daytime and at night, each was tagged with a small piece of radioactive tantalum (Ta^{182}) whose radiations enabled the observer to find the insect in the wheat and in the soil from about 40 cm with a portable Geiger-Müller probe.

CONDITIONS OF THE EXPERIMENTS AND METHODS USED

Conditions of the Wheat

The area selected to study the behaviour and movements of individual insects during their intensive feeding period in early June was a large wheat field (Field I) close to the village of Palasht which is about 2 km from Mamazan and about 20 km south-east of Tehran.

The wheat was approaching maturity, but in the main part of the field most of the leaves and stems were still green and succulent and the grains were in the "milky-white" stage of development; only a few ears were ripe and ready to harvest. Here the stems were taller (mean height, 82 cm) and more densely sown (86 stems per 0.25 m^2); this part of the crop is referred to as "closed wheat" (Fig. 1, left). At one end of the field where the wheat was shorter (mean height, 62 cm) and sparsely sown (42 stems per 0.25 m^2) and therefore referred to as "open wheat", the leaves and stems were dry and the grain mature (Fig. 1, right). Because of these differences and of possible differences in temperature and humidity, we decided to study the individual behaviour of three bugs in each of the two parts of the wheat crop separately.

During the spring and summer the field had been irrigated several times, but irrigation was less frequent in 1960 than in previous years because of a shortage of water. When work started on 6th June, the field had not been irrigated for at least one month, and the soil in the open and closed wheat areas was hard and dry with many deep cracks. The open wheat, being on slightly higher ground, was not so well irrigated as the main part of the field and the plants did not grow so well there. Work had to be discontinued when the field was flooded in the evening of 10th June.

At the end of June, another wheat field (Field II), 1 km to the west of the first, was selected to study the behaviour at the end of the insects' feeding period. This crop, like all the wheat throughout the area, was mature and ready for harvest. All the stems and leaves were dry and brown and the only green vegetation consisted of several kinds of weed, mostly *Alhagi camelorum* Fisch. ("Camelthorn"), *Glycyrrhiza glabra* L., a few vetches (*Vicia spp.)* and some *Prosopis stephaniana* Kunth., which were common in the crop. The wheat was as densely sown and as tall as that of the closed wheat at Field I. Field II had not

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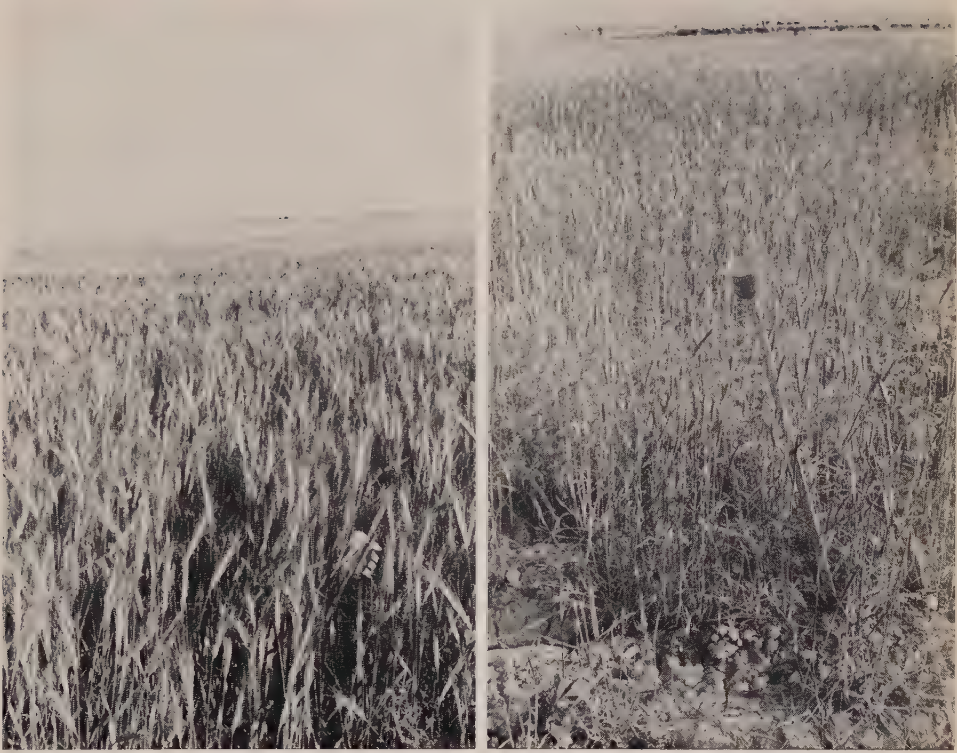

Fig. 1. Views of the "closed" wheat (left) and "open" wheat (right) at Field I, Palasht. The marker stick in the open wheat is 1 m long; the deep cracks in the soil are visible.

been irrigated for about six weeks and the soil was hard and dry and extensively cracked.

Microclimatic Records

In Field I records were kept of the temperature (°C) and relative humidity of the air at two stations, one in the closed wheat and the other, 100 m away, in the open wheat. At each station two thermographs, protected from the sun, were set up to record temperatures at ear level and at ground level. The two instruments in the open wheat also recorded relative humidity. The heat-sensitive elements of the instruments were about 80 cm (closed wheat) and 60 cm (open wheat) above ground for the ear level records, and about 10 cm above the soil for the ground level records.

An Assmann hygrometer (whose thermometers could be read to 0.5 °C) was also used to record air temperatures and humidities at the same levels as the thermographs at both stations for each hour of the day between sunrise and sunset. No records were made of wind speeds or of the temperatures and humidities in cracks in the soil.

At Field II, not only were air temperatures recorded at ear level and at ground level but the direction and speed of the wind were also measured several times each day with a cup anemometer set up in the open about 5 m from the edge of the wheat. The cups of the instrument were about 80 cm above ground level.

Labelling

At both fields, six young adult bugs recently collected from wheat were each labelled with a small piece of radioactive tantalum (BANKS, 1955). To avoid the use of an anaesthetic each bug was immobilised in a cell cut into a block of wood, its head being covered by a piece of sheet cork fixed to the wood by a pin. The tantalum label was then attached to the posterior part of the insect's scutellum with a spot of quick-drying cellulose paint which served as an additional means of identifying the insect. The tantalum labels, cut from an irradiated strip, were approximately 0.05 mm × 0.16 mm × 0.46 mm in size, of average weight 0.06 mg and of average specific activity 8 μc (120 μc/mg). A young adult bug had an average weight of 11 mg and was 12 mm long and 7 mm wide. The tantalum label was attached about 1 mm from the posterior right-hand edge of the scutellum. Insects labelled in this way were readily located in the wheat with a portable Geiger-Müller tube at distances up to 40 cm. They suffered no apparent harmful effects during the experiments, which lasted no more than four days.

As soon as the paint was dry, the insects were taken to the field where they were set free on to wheat ears. Their initial positions were marked by a stake in the ground and subsequent positions by small numbered tags attached to the wheat stems by sticky tape or to short pegs when the insects were in the soil.

The bugs were observed every half-hour from sunrise to sunset and on several occasions after dark, for the three or four days of the experiment at Field I, but less frequently at Field II. Distances between positions in which the bugs were found, the directions of one position from another and the heights of the insects above ground level were recorded. Details recorded of the bugs' behaviour included their orientation to the sun and to the plant, whether they were feeding and on what part of the plant they were situated, and whether they were moving when observed.

RESULTS

Microclimatic Conditions in the Wheat

Throughout June the weather was hot and dry with bright sunshine nearly all day. There had been no rain for many weeks. The air was usually still at sunrise (about 0500 h) and at sunset (about 1900 h) but each day a strong hot southerly wind arose at about 1000 h and lasted to about 1600 h.

The microclimatic records, in so far as they are of interest to the study of the behaviour of the bugs, are summarised in Fig. 2 which shows the average temperatures and relative humidities of the air taken with the Assmann hygrometer at ear level and ground level in both parts of Field I. Minimum temperatures recorded by the thermographs occurred between 0300 and 0500 h and maximum temperatures between 1300 and 1500 h daily. Temperatures were higher at ground level than at ear level during the middle of the day; humidities were

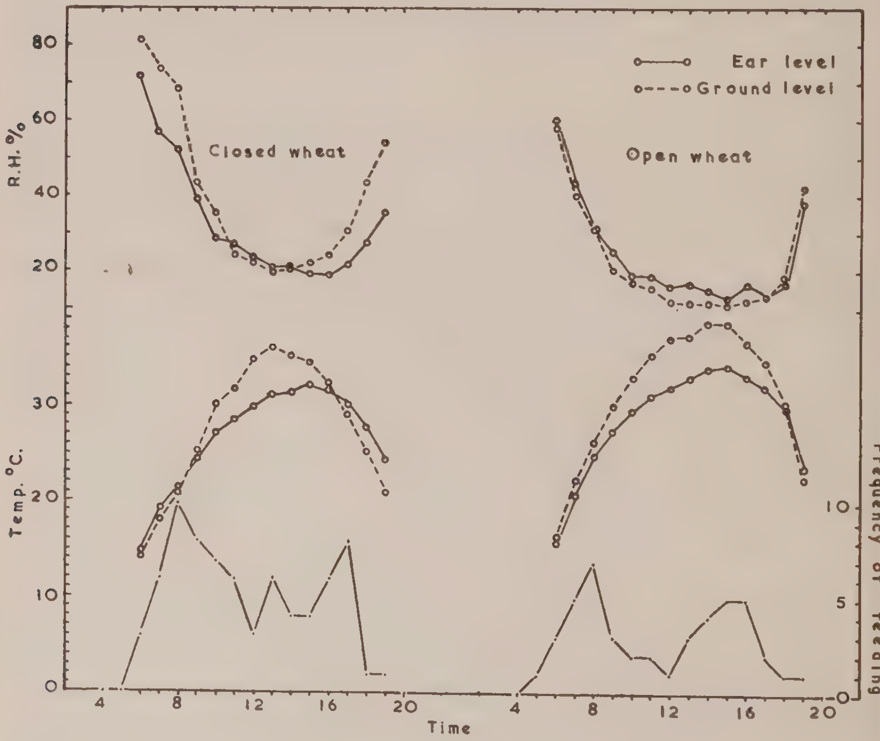

Fig. 2. Frequency of feeding of 6 Sunn Pest in the "closed" wheat and "open" wheat at Field I, with mean hourly temperatures and relative humidities of the air at ear level and ground level.

higher at ground level than at ear level during the night in the closed wheat but varied very little in the open wheat. The air in the open wheat was hotter and drier than in the closed wheat. At ear level in the wheat in Field II at the end of June, the maximum daytime temperatures were about 3° higher, and the night minima about 8° higher, than at Field I at the beginning of the month.

Horizontal movements

On the average, the labelled insects changed their positions about four times a day and moved about 60 to 70 cm each day. Those in the open wheat moved more often and covered somewhat longer distances than the bugs in the closed wheat (Table I, rows 2 and 3). After three days the distances of the bugs from their release points averaged 143 cm (57 in.) and the maximum recorded was 226 cm (90 in.). Fig. 3 shows the positions in which the insects were found and that they sometimes moved relatively long distances in one direction; such movements were not related to time of day, to the direction of the sun or to an earlier position of the insect on a plant or in the soil. There was no common direction of movement, which was changed apparently at random.

TABLE I

Summary of observations on the behaviour of Sunn Pest in Field I (early June) and Field II (late June), Palasht, Iran

	Field I		Field II
	Closed wheat (3 insects)	Open wheat (3 insects)	(6 insects)
1. Total observations	239	194	88
2. Mean no. of positions per day	3.8	4.7	3.2
3. Mean distance travelled per day (cm)	60	73	70
4. Mean duration in soil per day (h)	0.6	7.8	14.0
5. Mean proportion of time spent on wheat ears (%)	80	41	—
6. Feeding occasions/total observations (%)	35	25	11
7. Occasions when bugs had opportunity to feed/total observations (%)	83	51	42
8. Feeding occasions/occasions when bugs had opportunity to feed (%)	43	49	25
9. Occasions when bugs were feeding in the sun/total feeding occasions (%)	86	60	20
10. Occasions when bugs were feeding with the head downwards/total feeding occasions (%)	72	57	60

These results suggest that the insects feed and live in a restricted area of wheat, a conclusion supported by the results of the two following experiments made at Karimabad, 10 km from Field I.

1) On 22nd June, an area 12 m square in a large wheat field was divided into 4 plots of 6 m square. Bugs were swept from the plots and each insect was marked on the scutellum with a spot of paint to denote the plot from which it had been collected. Altogether, 727 insects were marked and distributed evenly in the appropriate plots, care being taken to ensure that enough were put along the margins of the plots and that no bugs were released into adjacent plots.

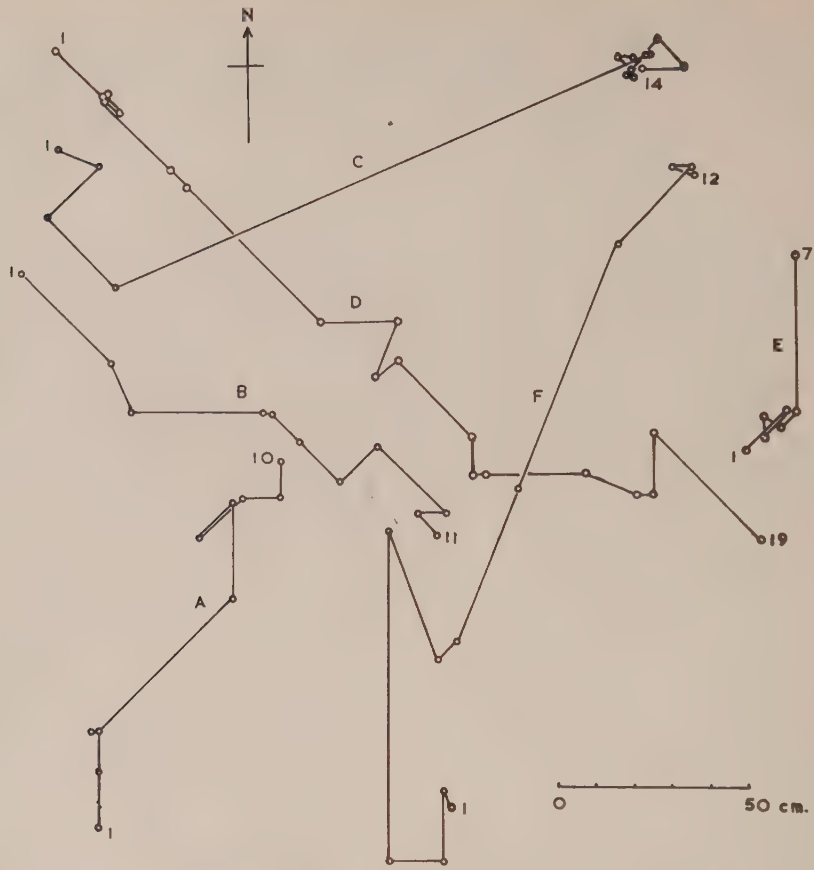

Fig. 3. Horizontal movements of 6 Sunn Pest (A-F) at Field I. Circles indicate the positions at which the insects were found during 3—4 days; numbers indicate the first and last positions.

Two days later and on 8 more successive days bugs were collected from each plot by sweep-net. Table II shows that nearly all marked bugs were recaptured in the plots in which they had been caught and liberated on 22nd June. In 10

TABLE II

Summary of recaptures of Sunn Pest marked with paint and liberated on four adjacent plots of wheat in a field at Karimabad, Iran on 22nd June 1960

	24.VI	25.VI	26.VI	27.VI	28.VI	29.VI	30.VI	1.VII	2.VII	Totals
Number recaptured in plot where originally released	103	45	11	9	3	2	2	2	1	178
Number recaptured in adjacent strip 1 m wide of neighbouring plot	7	7	3	0	0	1	0	0	2	20
Number recaptured in other plots, not in adjacent 1 m strip	1	0	0	1	0	0	0	0	1	3

days only 23 of a total of 201 recaptured marked bugs were found in a plot in which they had not been liberated, and all but 3 of these were in a strip 1 meter wide adjacent to the plot in which they had been released.

2) On 18th June, 3540 bugs, swept from a plot 23 m square in a large field of wheat, were marked individually with paint and liberated where they had been caught. Two days later, when the surrounding wheat was swept for a distance of 2 m from the edges of the plot, only 4 marked bugs were found in a collection of 2500 insects.

Vertical Movements

The radio-isotope-labelled insects in the closed wheat spent nearly the whole time above ground, usually on the wheat ears, but the bugs in the open wheat spent considerable time in cracks in the soil (Table I, row 4). Air temperatures above ground in the open wheat, where there was little shade, were higher than in the closed wheat and may have been too high for the bugs. They stayed for long periods in the cracks in the soil where, presumably, it was cooler and more humid.

There was considerable variation between the movements of the individual bugs but, on the average, those in the closed wheat spent 80 per cent and those in the open wheat 40 per cent, of the time on the wheat ears (Table I, row 5). In both habitats, the bugs were often on the wheat ears in the early mornings, less often at mid-day and again more often in the mid-afternoon (Fig. 4), a periodicity which is less pronounced than that of their feeding activity (see below). The insects did not move much during the night, for, of the total 21 observations of individual insects in the early morning, in 15 they were found in the positions which they had occupied at sunset the day before, no matter whether the position was in the soil or on a wheat ear.

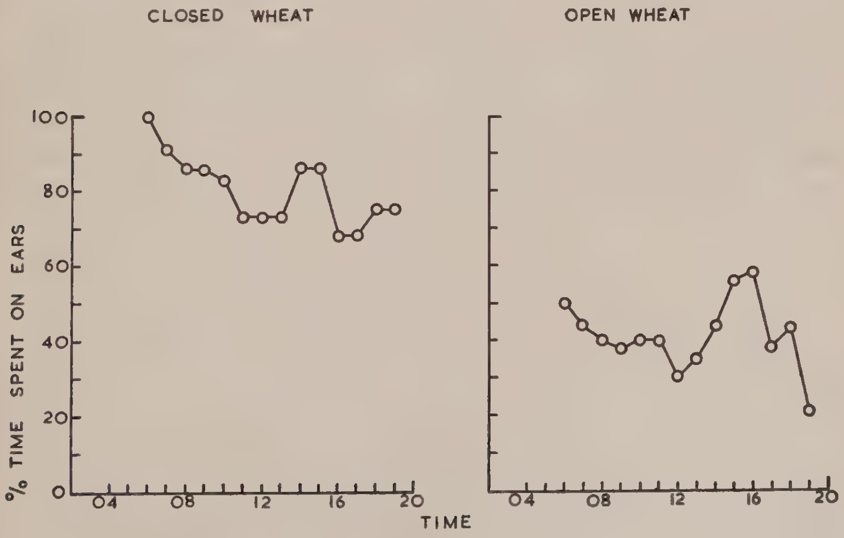

Fig. 4. Proportion of time spent on the wheat ears by 6 Sunn Pest in the "closed" wheat and "open" wheat at Field I.

Feeding

An insect was recorded as feeding when the rostrum was lowered and its tip applied to the plant surface in the typical feeding attitude. Because the bugs in the closed wheat spent most of the time on the plants, they had many more opportunities of feeding than those in the open wheat which spent much of the time in the soil (Table I, row 7). Feeding occurred more often in the closed wheat (35 per cent of all the observations) than in the open wheat (25 per cent of all the observations) (Table I, row 6), but when the bugs in the open wheat were on the plants, they fed more often than those in the closed wheat (row 8).

The insects did not usually start to feed until direct sunlight reached them during the early morning; later in the day, they usually moved themselves on the wheat ears so that the sun's rays shone directly on to their backs. Of all the occasions when the insects were seen to feed, 86 per cent (closed wheat) and 60 per cent (open wheat) were when the insects were in direct sunlight (Table I, row 9). Figure 2 shows that the bugs had a period of morning feeding activity which started at sunrise and rose to a peak at 0800 hours, followed by a decrease with a minimum at mid-day, and succeeded by an afternoon period of feeding with a maximum around 1600 h; feeding almost ceased at sunset at 1900 h. The bugs in both habitats showed this pronounced periodicity in feeding, which was apparently not directly related to temperature (see discussion below). The insects were located after dark and observed by lamplight on two successive nights; on the first night, at 2030 h, four of the six were feeding on wheat ears and on the second night, at 2330 h, three were feeding. Although active movements generally ceased after sunset, feeding continued for a time at least but ceased completely by early morning.

In 72 per cent (closed wheat) and 57 per cent (open wheat) of the occasions when feeding was recorded, the bugs were feeding on the ears with their heads downwards (Table I, row 10).

The bugs in the closed wheat fed only on the ears of wheat, but on three occasions bugs in the open wheat were seen feeding on wheat stems, although the plants were yellow and dry and the ears ripe. Several kinds of weed, mainly *Convolvulus* spp., *Prosopis stephaniana* and *Alhagi camelorum*, were common in the closed wheat but the bugs were never seen feeding on them. In contrast, at a wheat crop near Karimabad during the following week, *Eurygaster integriceps*

TABLE III

Distribution of Sunn Pest in 49 quadrats (1 m²) in relation to the distribution of the weeds Prosopis stephaniana *and* Alhagi camelorum

No. of Sunn/quadrat	No. of quadrats: without weeds	with weeds
0	7	0
1— 4	6	3
5— 9	6	11
10—19	1	12
20—39	0	2
40—79	0	1
Total quadrats	20	29
Total Sunn	63	338
No. Sunn/quadrat	3.15	11.65

were often seen feeding on *P. stephaniana* and *A. camelorum*. At this field 49 quadrats, each one meter square, were examined thoroughly for Sunn Pest in a plot 23 m square. The distribution of the insects in the plot was clearly associated with that of the two weeds (Table III), and the density of the insects was nearly four times greater in the quadrats where one or both species of weed occurred. The wheat there was dry and ready for harvesting and the bugs fed on the weeds, presumably, to obtain water which they could not get from the wheat. This crop was in the same ripe condition as that of the open wheat at Field I at Palasht where, had weeds been common, the bugs would probably have fed on them to obtain water. In the closed wheat, however, the wheat plants, being unripe, probably provided adequate water for the insects which, therefore, did not feed on weeds.

Behaviour at the end of the Feeding Period

The behaviour of another six young adults, tagged with tantalum labels, was studied in Field II at the end of June when the insects had nearly finished their feeding. Most Sunn Pest had migrated from the wheat fields and the six insects were observed for 3—4 days until they disappeared, presumably having also flown away. At this time, air temperatures were considerably higher than at the beginning of the month; at ear level, the air temperatures at sunrise and sunset were 8—9° higher and the daytime maximum was about 3° higher than they had been in Field I. The wheat was completely dry and the grains hard and ripe.

The bugs behaved very differently from those at Field I, for they spent very little time feeding and stayed for long periods in cracks in the soil, even longer than those in the open wheat of Field I (Table I, row 4). Table I shows that feeding was observed in only 11 per cent of the observations made, and that when bugs were seen on plants and had an opportunity to feed (42 per cent of all observations), in only one quarter of the occasions was feeding observed (row 8). One bug was not seen to feed at all although it was always found on the plants.

Although these bugs spent more time in the soil and fed less often than those in Field I, they changed their positions almost as often and moved about the same average distance each day. Although only one bug was seen to feed on the stem of *Convolvulus* sp. and another to feed on a vetch stem (*Vicia* sp.), bugs were found on weeds on 12 occasions and feeding on weeds may have been more common than in Field I.

DISCUSSION

ACHARD & ADLE (1927), working in Iran, failed to confirm an observation in Syria that the insects spend the cool hours of the morning and evening on the plants and that they shelter under the plants or in cracks in the soil during the heat of the day, an opinion often held by others (HIBRAUOI, 1930; LOGOTHETIS, 1956; YÜKSEL, 1958). JOURDAN (1955) says that in Morocco the bugs ascend the wheat stems only between 1000 and 1700 h; he studied various species of *Eurygaster*, other than *E. integriceps*, but the discrepancy between his observations and those of others is probably to be explained by differences in the daily climatic cycle. The behaviour of the insects is affected by local weather conditions: PEREDEL'SKII (1947), working in south-western Uzbekistan and in northern Kirghizia, describes vertical movements of *Eurygaster integriceps* in March and May similar to those described by Hibrauoi, Logothetis and by Yüksel; but from May onwards, he says, when the ears are forming on the wheat and temperatures are not less than 20° during the night and are over 30° during the day, the bugs remain constantly on the plants and feed on the ears even at night; he observed that the bugs do not avoid exposure to the sun, even during the hottest hours. Our results, which agree with those of Pederel'skii, are opposed to those of MAKHOTIN (1947b) who reports that during the first half of the day the insects descend towards the ground and then spread "horizontally". In the second half of the day, they move from the ground to the tops of the plants.

Our observations in Iran indicate a periodicity of vertical movement which is correlated with an even more pronounced periodicity in feeding activity. The bugs in the open wheat showed a periodicity in their vertical movements more strongly than those in the closed wheat which spent nearly all the time on the wheat ears. The extent of vertical movement seems to depend, therefore, on the state of the wheat crop and on local conditions. Our results show that the insects' horizontal movements were slight, under the conditions when the observations were made, the average distance covered in the different stations ranging from 60 cm to 73 cm per day. The various experiments indicate that widespread lateral movement, and therefore rapid mixing, do not normally occur among newly-adult Sunn Pest.

The young adult Sunn Pest which we studied fed often on the wheat ears to build up the food reserves needed for migration and overwintering, but they did not feed continuously although they were often on the plants; our records show that less than half their time on the plants was spent in feeding.

Although feeding continued after dark at a very reduced rate, for a time at least, other activity almost ceased; after sunset, the insects nearly always stayed were they happened to be, whether they were on a plant or in the soil. Temperatures after sunset were as high as those in the early morning when feeding was often seen and, therefore, the almost complete cessation of activity at sunset was not caused by the fall in temperature alone but was probably associated also with the onset of darkness. Light is known to affect the insect's behaviour: for example, after release from the cell in which they had been labelled with the radioisotope, they pushed the covering watch-glass along the table towards the light, but they remained motionless when the watch-glass was covered with the lid of a tin; furthermore, the insects orientated themselves towards the sun when feeding on the wheat ears.

There is no direct correlation of the insects' feeding activity with the air temperatures recorded. The daytime temperatures were probably always well above the minimum when feeding can occur; between 0300 and 0500 h, the air temperature fell to 12°—14° at ear level and to 11°—14° at ground level, and at these temperatures feeding and other activity were probably decreased, only to increase again when the temperature rose to about 14° and direct sunlight reached the insects the next morning. MAKHOTIN's (1947a) laboratory experiments on the reactions of *E. integriceps* to temperature showed that the insects become increasingly active from 6°—12° to 15°—30°; between 25° and 37° they become inactive and from 37°—48° another maximum of activity ("pathologic activity") occurs before they die at 49.5°. Although our field observations do not fully agree with Makhotin's findings, the falling-off in feeding activity during the hottest part of the day may have been caused by the rise in temperature into a range in which the insects become inactive, as Makhotin described. The periodicity of feeding with two peaks of diurnal feeding activity can not be satisfactorily explained until experiments are made throughout the night during summer to find any relationship between feeding activity and temperature without the complication of the light factor; similar observations should be made in the spring when temperatures are lower. Our field observations suggest that, under certain conditions at least, green weeds may supply an auxiliary source of food, or water, for Sunn Pest in cereal crops.

Our observations indicate that the general behaviour of young adult Sunn Pest on the wheat changes before migration. After the last moult, the insects spend much time in feeding (when conditions are favourable, as they were in the closed wheat in Field I) and spend very little time in the soil. When feeding ceases, the insects descend to the soil where they stay for a long time until they are ready to fly away. This pattern of behaviour is probably associated with the extent of accumulation of food reserves, the factor which is undergoing the greatest change at this time; the female gonads remain immature until the following year. The building-up of the reserves is itself influenced apparently by such local conditions as the state of development of the wheat and the microclimatic conditions in the crop, and by the presence of green weeds on which the insects can feed.

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RESUME

ÉTUDES DANS LES CHAMPS DE L'ACTIVITÉ DIURNE ET DU COMPORTEMENT ALIMENTAIRE DU "SUNN PEST" EURYGASTER INTEGRICEPS *PUT. (HEMIPTERA, SCUTELLERIDAE) SUR BLÉ DANS LE NORD DE L'IRAN.*

1. Le comportement individuel de six jeunes adultes de la punaise des céréales a été étudié dans un champ de blé dans le Nord de l'Iran, durant des périodes de 3 à 4 jours, au début de la période d'alimentation des adultes, début juin. On a étudié six autres insectes à la fin juin quand l'alimentation est presque terminée et quand les insectes sont prêts à émigrer vers les montagnes. Chaque insecte était marqué avec un petit morceau de Ta^{182} de sorte que sa position sur le blé ou dans le sol pouvait être repérée (ou relevée) toutes les demiheures du lever au coucher du soleil.

2. Au début de la période d'alimentation, les insectes dans une partie du champ où le

blé était semé dense, où les feuilles et les tiges étaient encore vertes et les grains encore tendres, passaient la plupart du temps sur les plantes, et se nourrissaient fréquemment sur les épis de blé. Quand le blé était semé de façon éparse, le temps plus chaud et plus sec et les plantes sèches avec des grains mûrs, les insectes passaient beaucoup plus de temps dans des crevasses du sol et s'alimentaient moins souvent.

3. Les mouvements horizontaux des insectes étaient réduits à une petite surface de la culture. Les mouvements verticaux présentaient une périodicité qui était moins prononcée que l'activité alimentaire qui débutait après le lever du soleil, atteignait un maximum à 8 h, déclinait rapidement jusqu'au milieu de la journée et remontait à un autre maximum dans l'après-midi. L'alimentation cessait presque au coucher du soleil, et les mouvements actifs étaient rares dans l'obscurité. L'activité alimentaire durant le jour n'était pas en corrélation directe avec les températures de l'air relevées. La distribution de la punaise des céréales dans une culture de blé sèche et prête pour la moisson était en relation étroite avec celle de deux espèces de plantes sauvages vertes sur lesquelles on a vu les insectes s'alimenter.

4. Vers la fin de la période alimentaire, le comportement des insectes était différent: ils s'alimentaient rarement et passaient de longues périodes dans des crevasses du sol où ils se déplaçaient activement.

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STARVATION AND DESICCATION IN TSETSE FLIES *(GLOSSINA)*

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Collections of tsetse flies were made from different regions and at different seasons, and their fat and water contents determined. The results showed that of the two potential causes of death, starvation is far more important than desiccation.

Since the demonstration by NASH (1933) of a negative correlation between saturation deficit and the apparent density of tsetse flies, desiccation has been considered an important cause of death in natural populations (eg. JACKSON, 1944; NASH & PAGE, 1953; PAGE, 1959). But results of laboratory studies on the water balance of tsetse flies do not support this view (BURSELL, 1959, 1960) nor has any other evidence been adduced in its favour. Quite apart from its intrinsic interest the concept that desiccation may play a part in the regulation of tsetse numbers is of relevance to the theory and practise of discriminative clearing (eg. PAGE, 1959) and it would clearly be desirable to base it on somewhat firmer ground than the original correlation coefficient can be considered to furnish.

A study of the physiological state of tsetse flies caught in the field might answer this purpose, but complications are introduced by the strong sampling bias which characterises standard methods of collection (BURSELL, 1961). Such methods tend systematically to exclude a large proportion of the hungrier flies, and it is just these which would be of relevance to the problem of mortality. This difficulty may, however, be overcome by considering the relation between the two types of reserve, food and water, in individual flies within the range sampled. Both decrease during later stages of the hunger cycle, and it should be possible to extrapolate a plot of one against the other and determine which would be first depleted to the critical level. With this type of analysis it would not matter that certain sections of the hunger spectrum fail to be represented in their correct proportions provided only that a few representatives are caught at extremes of the range so that extrapolation may be made with some degree of confidence. The method was accordingly applied to collections of tsetse flies made in different parts of East Africa during 1958 and 1959.

MATERIAL AND METHODS

Males of *Glossina swynnertoni* Aust. and *G. morsitans* Westw. were caught by standard catching parties in the localities listed in Table I. Fat contents and water contents were estimated according to methods described elsewhere (BURSELL, 1959). To avoid complications due to water present in the residual blood meal, the water content of the thorax was taken as an indication of the state of tissue hydration.

In what follows fat content will be used as a measure of the quantity of food reserve possessed by a fly; the contribution made by other substances, in particular by the remains of the blood meal still present in the gut, has not been taken into account and it is necessary to enquire whether this omission is likely to affect the results substantially. Errors arising have in the first place been minimized by restricting analysis to flies in the last stages of the hunger cycle (stages III and IV of JACKSON, 1933); in these the amount of blood remaining in the gut is small in relation to the total amount of fat (cf. Table I, columns 7 and 11). In fact it has been shown that the proportion of residual blood meal to fat decreases progressively with time to reach values of less than 10% in late stage IV (see BURSELL, 1961 Fig. Id). When it is further considered that the high nitrogen content of the blood meal must make it a far less effective source of energy, weight for weight, than fat, it is clear that the error involved in excluding it from analysis is likely to be negligible.

To allow for differences in size between individual flies regressions were first of all calculated of thoracic water content on size and of total fat content on size for each sample (see Table I, columns 7—10). The fat and water contents of individual flies were corrected on the basis of these regressions to give "size-specific" values, that is, the amount of water or fat which a fly would have been expected to contain had its size been equal to the mean size of flies in the sample. The "size-specific" water content was then plotted against the "size-specific" fat content, and examples of such plots are shown in Fig. 1 a and c. The regression of water content on fat content was calculated and the regression line drawn.

The critical level of fat and water contents for nonteneral flies was determined by collecting stage III flies in the field and starving them at high and low humidities respectively; fat and water contents were determined at the time when flies were still able to respond to mechanical stimulation but incapable of performing the "righting" reaction. This point was reached when fat had been reduced to 6.4% of total dry weight or when water had been reduced to 64.9% of the non-fatty wet weight1). The regressions of fat on water were extrapolated to the critical level of fat content to give the amount of water remaining when fat reserves have been reduced to zero. The difference between this amount and the critical water content for a fly of the appropriate size would then give the quantity of water reserves remaining when fat reserves have been totally depleted.

The procedure was repeated with fat content as the "dependent" and water content as the "independent" variable, to give the quantity of fat reserves remaining when water reserves have been reduced to zero (see Fig. 1 b and d).

This treatment of the data rests, of course, on the assumption that the relation between fat content and water content is a rectilinear one, FISHER's (1948) test of straightness of regression lines was applied, but in none of the samples could

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*) The values are slightly different from those previously reported for teneral flies (BURSELL, 1959).

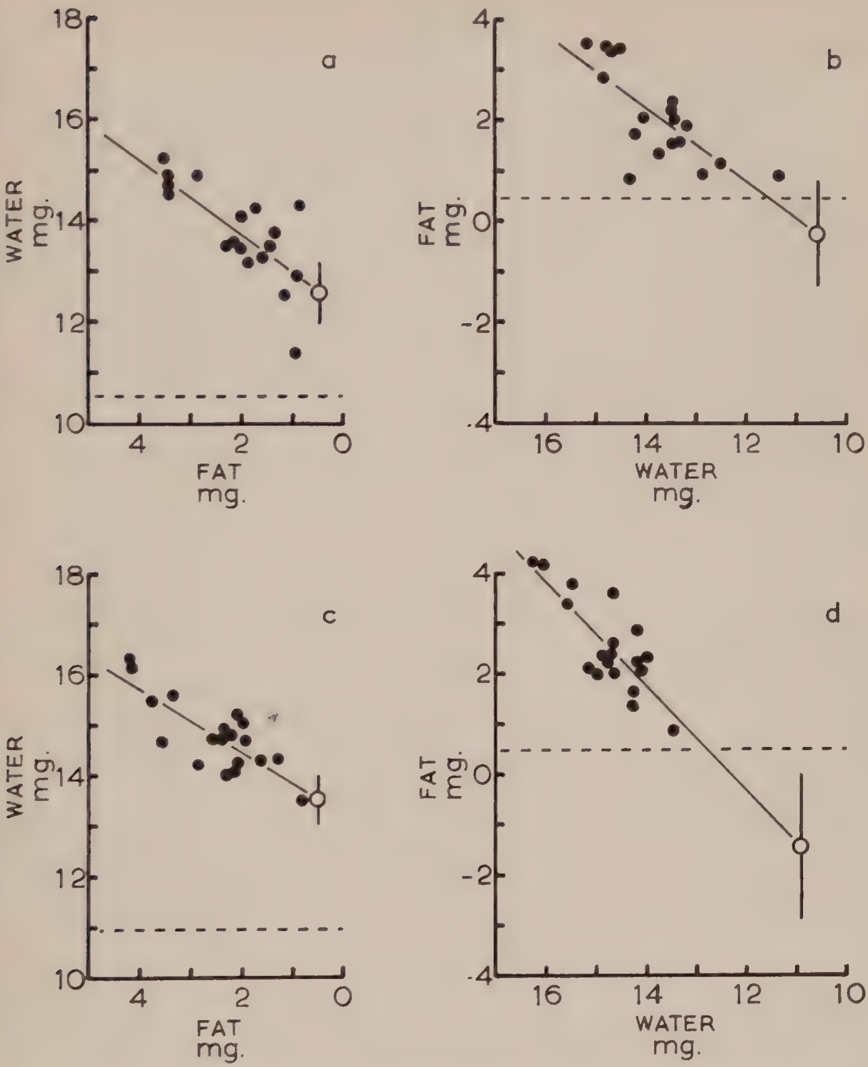

Fig. 1. The relation between fat and water contents for collections of tsetse flies made in the dry season.

a and b — *G. swynnertoni;* Shinyanga; July, 1958.

c and d — *G. morsitans;* Urambo; September, 1958.

The open circles with vertical lines represent estimates of the amount of one reserve (± twice its standard error) when the other is zero; the critical level of reserves is shown as broken lines. For further explanation see text.

a significant departure from rectilinearity be demonstrated. Departures may occur, as indeed they could be expected to, but this statistical analysis shows them to be small in relation to the variance of the observations, and they would therefore not be expected to affect conclusions which must be drawn from effects which are large in relation to sample variance.

TABLE I

The water and fat reserves of tsetse flies

1	2	3	4	5	6	7	8	9	10	11
Locality	Month %	$\overline{\text{Relative Humidity}}$	$\overline{\text{Temp.}}$ °C	$\overline{\text{Sat. Deficit}}$ mm Hg	$\overline{\text{Thoracic Surface}}$ arb. unit	$\overline{\text{Fat}}$ mg	b_f	$\overline{\text{Water}}$ mg	b_w	$\overline{\text{RBM}}$ mg
I. *G. swynnertoni*										
Shinyanga	July	45	22.3	11.1	7.79	2.13	0.62	13.65	1.94	0.49
,,	Aug.	54	22.5	9.4	7.82	2.09	0.65	13.82	2.41	0.45
,,	Sept.	49	25.1	12.2	7.50	2.25	0.23	13.53	2.44	0.56
,,	Oct.	41	25.9	14.8	7.37	2.60	0.26	12.91	2.22	0.50
,,	Nov.	61	25.0	9.2	7.04	2.37	0.50	12.23	2.05	0.55
,,	Dec.	79	21.4	4.0	7.02	1.99	1.27	11.86	2.71	0.46
,,	Feb.	80	23.8	4.4	7.79	3.10	0.56	13.99	2.11	0.57
II. *G. morsitans*										
Ngara	June	80	20.1	3.5						
Control					8.90	2.80	0.57	15.75	1.82	0.78
Cleared					8.74	1.93	0.23	16.11	2.37	0.60
	Sept.	50	21.2	9.4						
Control					8.40	3.37	0.28	15.10	2.18	0.79
Cleared					8.49	2.82	0.60	15.14	2.30	0.71
Urambo	Sept.	38	25.2	14.9						
Control					8.31	2.36	0.44	14.71	1.48	0.57
Cleared					8.48	3.01	0.02	15.31	1.86	0.73
,,					8.30	3.30	0.76	14.90	2.25	0.71
Ankole	Dec.	73	19.8	4.7						
Control					8.94	2.84	0.92	16.05	2.32	0.83
Cleared					8.73	2.55	0.84	15.60	2.19	0.78
,,					8.80	2.73	0.84	15.56	2.48	0.73
,,					8.66	2.03	0.65	15.01	2.23	0.75
Geita	Oct.	52	23.4	10.4						
Cleared					8.44	2.78	0.57	14.04	2.11	0.55

Mean values are denoted by a line above, eg. $\overline{\text{Fat}}$ = mean fat content

Thoracic surface in mm² = 0.744 (arb. unit)

b_f = coefficient of regression of fat on thoracic surface

b_w = coefficient of regression of water on thoracic surface

RBM = residual blood meal

RDW_{th} = thoracic residual dry weight

$\hat{\text{Water}}_{\text{fat}_0}$ = best estimate of water content when fat reserve is zero

$\hat{\text{Fat}}_{\text{water}_0}$ = best estimate of fat content when water reserve is zero

$b_{w/f}$ = coefficient of regression of water on fat

n = number of flies analysed

TABLE I (cont.)

12 $\overline{\text{DW}}_{\text{th}}$ mg	13 $\widehat{\text{Water}}\text{fat}_{\text{o}}$ mg	14 $\dot{\text{R}}\text{eserve}_{\text{w}}$ mg	15 t	16 $\widehat{\text{Fat}}_{\text{water}_{\text{o}}}$ mg	17 $\text{Reserve}_{\text{f}}$ mg	18 t	19 $b_{w/f}$	20 n
.70	11.98 ± .35	1.44	4.1	0.87 ± .26	0.40	1.5	1.00 ± .21	40
.73	12.58 ± .32	2.04	6.4	—0.26 ± .57	—0.73	1.3	0.74 ± .17	18
.38	12.10 ± .54	2.16	4.0	1.14 ± .41	0.70	1.7	0.79 ± .28	27
.17	11.71 ± .43	2.12	4.9	1.23 ± .47	0.80	1.7	0.58 ± .19	31
.88	10.27 ± .51	1.32	2.6	1.13 ± .34	0.73	2.1	0.95 ± .25	27
.95	11.33 ± .25	2.38	9.5	0.47 ± .67	0.07	0.1	0.32 ± .14	28
.61	13.20 ± .32	2.81	8.8	1.61 ± .60	1.12	1.8	0.30 ± .12	48
6.64	13.89 ± .34	1.61	4.7	0.79 ± .37	0.25	0.7	0.82 ± .14	40
6.41	14.86 ± .26	3.05	11.7	—0.74 ± .46	—1.27	2.8	0.84 ± .14	35
6.23	14.46 ± .38	2.92	7.7	2.17 ± .67	1.66	2.5	0.22 ± .14	41
6.19	14.03 ± .24	2.59	10.8	—0.01 ± .56	—0.53	0.9	0.49 ± .09	49
5.92	13.52 ± .25	2.61	10.5	—1.46 ± .73	—1.95	2.7	0.63 ± .11	19
6.28	14.01 ± .46	2.36	5.1	1.78 ± .42	1.26	3.0	0.53 ± .17	46
5.98	13.64 ± .32	2.59	8.1	0.67 ± .63	0.18	0.3	0.46 ± .11	44
6.74	14.84 ± .24	2.44	10.0	0.30 ± .51	—0.25	0.5	0.48 ± .10	50
6.60	14.73 ± .17	2.59	15.0	—0.19 ± .52	—0.73	1.4	0.42 ± .07	66
6.52	14.64 ± .22	2.58	11.7	—0.09 ± .57	—0.63	1.1	0.43 ± .08	52
6.40	14.59 ± .27	2.70	10.0	0.16 ± .71	—0.37	0.5	0.24 ± .14	19
6.00	12.08 ± .37	1.03	2.8	0.22 ± .48	—0.27	0.6	0.81 ± .14	16

Meteorological observations were made concurrently with the collections using a regularly checked thermohygrograph; the figures included in Table I represent the means of 2-hourly values for the week in which samples were taken.

RESULTS

A summary of the results is shown in Table I. Regular samples of *G. swynnertoni* were taken from the beginning of the dry season (July, 1958) till the middle of the rains (February, 1959). *G. morsitans* was collected from four different areas during 1958:

a) from Ngara (3° S.; 31° E.) where an open, *Afrormosia*-dominated woodland formed the tsetse habitat. Comparisons were made at two different seasons between areas which had been subjected to discriminative clearing four years previously and uncleared control areas. The clearings had resulted in very substantial reductions in apparent density but had failed to cause eradication.

b) from Urambo (5°S.; 32° E.) in the Central Province of Tanganyika where tsetse flies are associated with a *Brachystegia* woodland. Here again a comparison was made between areas in which fly densities had been drastically reduced by discriminative clearing some years previously and control areas outside the treated region.

c) from Ankole (1° S.; 31° E.) where the tsetse habitat was an open woodland dominated by *Acacia hebacladoides*. The effect of discriminative clearing in this area had been erratic, sometimes but by no means always resulting in a reduction in apparent density. Control samples were taken from an area about 20 miles south of the cleared region.

d) from Geita (3° S.; 32° E), an area of *Brachystegia* woodland which had been subjected to discriminative clearing some years before1).

From the point of view of the relation between fat and water reserves the data presented in Table I can conveniently be considered as a whole, since in this respect there seems to be no consistent difference between species, between different seasons or between different places. The quantity of water reserves remaining when fat reserves have been exhausted is shown in column 14, and the following column shows the probability, in terms of t, that this quantity is significantly different from zero. It is clear that substantial reserves remain in every single case. Column 17 shows the quantity of fat reserves remaining when water reserves have been exhausted; this is significantly greater than zero in only 3 out of 19 samples; it is negative in half of the remaining cases, significantly so in 2 samples, implying that critical fat contents would have been reached before exhaustion of water reserves. Thus in 2 out of 19 instances the data do not enable a decision whether the flies would be likely to die of starvation or desiccation; in all the others they would be expected to reach the critical limit of fat content before water reserves are depleted. The results thus leave very little doubt that of the two possible causes of death, starvation is far and away the most potent. And this appears to be so whether samples are taken at the height of the dry season or after the break of the rains, in untouched habitat or after discriminative clearings have decimated the populations.

It may be pointed out that such errors as may have been introduced by failure to take account of the residual blood meal would not affect this conclusion. Since the proportion of residual blood meal to fat decreases with time the rate of food utilization relative to the rate of water loss (ie. the slope of the lines in Fig.

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*) For further details of the collecting areas reference may be made to Annual Reports of the East African Trypanosomiasis Research Organisation (1953, 1954—5 & 1958) and of the Department of Tsetse Survey and Control, Tanganyika (1955 & 1958).

1 b and d) will, if anything, have been underestimated; in other words the quantity of water reserves remaining when food reserves have been exhausted would be in excess of present estimates.

One other point requires comment, with particular reference to the results for *G. swynnertoni* which are uncomplicated by differences in type of habitat. Column 19 shows that there is a tendency for more water to be lost per unit of fat consumed during the dry season (July to November) than after the break of the rains (December and February) in accord with expectation. But in spite of this the thoracic water content shows the most remarkable constancy; the values given in column 9 can be corrected for size by means of the regression coefficients in column 10, and for a thoracic surface of 7.5 the sequence of figures is 13.2, 13.1, 13.5, 13.2, 13.2, 13.2, 13.4 mg. The thoracic water reserve of a fly of this size is nearly 10 mg so that the difference between the highest and the lowest values amounts to no more than 4% of the total reserves; this despite an almost fourfold difference in the saturation deficit between the driest and the most humid month. These results provide a most striking illustration of the powers of water conservation exhibited by the tsetse fly, the mechanisms of which have been discussed in earlier papers (BURSELL, 1959, 1960).

It may be objected that neither starvation nor desiccation have in fact been shown to be important causes of death so that the extrapolation of regressions to critical levels is of purely formal interest. But consideration of the frequency distributions of water and fat reserves in flies collected in the course of this work will go some way to dispose of such an objection. For this purpose consideration has been limited to flies caught in their natural habitat, and Fig. 2a shows the percentage fat contents of such flies in relation to the critical limit at 6.40 ± 1.05%. Over 2% of the total sample falls within the range of lethal limits, and this fraction of the population may be thought of as subject to imminent death from starvation. Comparable proportions are clustered in class intervals close to the critical level, and since the feeding response starts at fat contents of about 24% (BURSELL, 1961) these flies must have been unsuccessful in their quest for food for several days before capture; it is probable that many of them would have been destined to remain unsuccessful for the much shorter time remaining to them. When in addition a sampling bias which excludes a large proportion of the hungrier flies has to be taken into account the results leave little doubt that starvation is indeed an important cause of death.

The corresponding values for water contents are shown in Fig. 2 b, and the distribution is clearly of quite a different type. The proportion of the sample included within the critical range (64.91 ± 0.63) is negligible, and few flies are found near to it. The conclusions reached on the basis of extrapolated data are thus confirmed by these frequency distributions, which suggest that starvation is a far more frequent cause of death than desiccation.

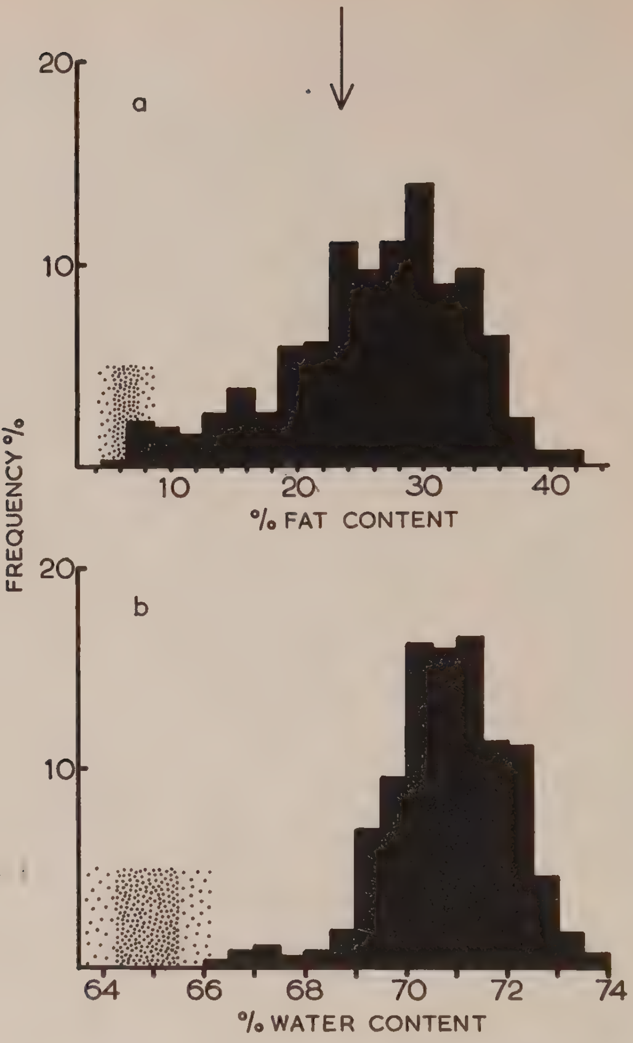

Fig. 2. The frequency distribution of fat (a) and water (b) contents for tsetse flies collected in their natural habitat.
Stippled areas show the critical ranges (the area within the standard deviation is heavily stippled, that within the limits of twice the standard deviation is lightly stippled).
The arrow marks the level of fat contents at which flies become ready to feed.

DISCUSSION

On the basis of present evidence and of earlier studies on the water balance of tsetse flies (BURSELL, 1959, 1960 & 1961) a strong case can clearly be made out against the view that desiccation is an important cause of death in natural populations of tsetse fly, at least as far as the savannah species are concerned. But this leaves the undoubted fact of the relation between tsetse densities and saturation deficit to be accounted for.

It may be that both saturation deficit and fly densities are correlated with a third factor or complex of factors, and that it is with this that a causal relationship should be sought. But to discuss at this stage other candidates for the role of limiting factor, such as temperature, would serve no useful purpose; while to attempt to define a complex of factors on the basis of correlation coefficients between tsetse densities and environmental conditions, taking due account of the complex interrelations between climate, vegetation and fauna which characterises the tsetse life history, would be a hopeless undertaking.

It seems that the problem must at this point be tackled from another angle; that only through a quantitative study of the contributions to mortality made by possible causes of death, like starvation and predation, can we hope to unravel the complex interplay of factors which finds ultimate expression in a correlation coefficient.

The collections on which the present study is based were made while under employment by the East African High Commission and my thanks are due to the Director of the East African Trypanosomiasis Research Organisation for permission to publish the results. I am grateful to the Director and staff of the Department of Tsetse Survey and Control, Tanganyika for facilities extended during my visits to Ngara, Urambo and Geita, and for placing at my disposal information on the history of the areas concerned; to Professor E. B. Edney for helpful criticism of the manuscript and to Professor B. H. Rasmussen who prepared the German summary.

ZUSAMMENFASSUNG

VERHUNGERN UND AUSTROCKNUNG BEI DER TSETSEFLIEGE (GLOSSINA)

In verschiedenen Gegenden und zu verschiedenen Jahreszeiten wurden Tsetsefliegen gesammelt und ihr Fett- und Wassergehalt bestimmt. Durch Extrapolation der Regressionen an Fett- und Wasserreserven konnten Schätzungen der nach Erschöpfung der Fettreserven übrigbleibenden Wasserreserven ermittelt werden, wie auch umgekehrt der übrigbleibenden Fettreserven nach Erschöpfung der Wasserreserven. Die Ergebnisse zeigten, dass von diesen zwei möglichen Todesursachen der Hunger viel wichtiger ist als das Vertrocknen. Dieses Ergebnis wurde durch eine Prüfung der Verteilung des Fett- und Wassergehalts bestätigt. Sie zeigte, dass sich eine beträchtliche Anzahl der Fliegen in der kritischen Zone des Fettgehalts oder sehr nahe daran befand, während nur ein geringer Bruchteil von ihnen einen Wassergehalt aufwies, der sich der kritischen Zone näherte.

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THE DETOXICATION ENZYMES CAUSING ORGANO-PHOSPHATE RESISTANCE IN THE HOUSEFLY; PROPERTIES, INHIBITION, AND THE ACTION OF INHIBITORS AS SYNERGISTS

BY

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A comparison of the breakdown enzymes in OP-resistant houseflies and the ali-esterase of susceptible flies shows differences as well as similarities. In general both are rapidly phosphorylated by the OP-compounds, but only in the case of the breakdown enzymes dephosphorylation can take place. The rate of phosphorylation of the breakdown enzymes by the toxicants is sometimes higher, sometimes lower than that of the ali-esterase with the same compound. Studies of the breakdown reaction showed the Km to be in the order of 10^{-6}—10^{-8} M, the turnover numbers to range from 0.05—0.7 per minute. Whether dephosphorylation takes place depends on the type of breakdown enzyme and the nature of the phosphoryl group. For instance, in the malathion-resistant strains only the dimethyl phosphorylated enzymes are dephosphorylated, but the dimethyl and dipropyl phosphorylated enzymes are not. Dephosphorylation of the enzymes of diazinon-resistant strains occurs with both dimethyl and diethyl, but not with dipropyl compounds. Consequently hydrolysis of some OP-compounds can be blocked by the addition of others that irreversibly phosphorylate the enzymes. Substances that can block the reaction have a marked synergistic action when sublethal doses are applied simultaneously with the OP-compound to which a strain is resistant. There is evidence that the breakdown enzymes can still hydrolyse methyl butyrate and some other esters although at a rate which is only a few percents of that of the ali-esterase. The problem as to how enzymes with a rather low breakdown capacity could confer high resistance is discussed.

It has been found that homogenates of organophosphate-resistant strains of houseflies show a very low esterase activity to simple esters such as methylbutyrate and phenylbutyrate, whereas in homogenates of strains that are not resistant to these insecticides a high esterase activity is present (van Asperen & Oppenoorth, 1959). This difference in ali-esterase activity is dependent on a difference in a single gene. The gene causing low activity has been shown to be responsible for resistance to organophosphates. A number of different alleles of this gene has been found, all causing low esterase activity, but differing in the kind of resistance which they confer to the strains (Oppenoorth, 1959b).

In the resistant strains detoxication enzymes are present (one in each strain) which seem to be produced under the influence of mutant alleles in the place of the ali-esterase which is made under the influence of the wild-type allele (van Asperen & Oppenoorth, 1960). The present paper deals with further studies on the properties of the detoxication enzymes, and further evidence is provided that they represent "mutant ali-esterases". The following is a full account of investigations which were briefly reported elsewhere (Oppenoorth & van Asperen, 1960 a and b).

MATERIALS AND METHODS

The following organophosphates have been used:

methyl paraoxon	O,O-dimethyl O-p-nitrophenyl phosphate
DDVP	,, ,, O-2,2 dichlorovinyl phosphate
chlorooxon	,, ,, O-3 chloro-4 nitrophenyl phosphate
malaoxon	,, ,, S-(1,2-dicarboethoxyethyl) phosphorothiolate
malathion	,, ,, S-(1,2-dicarboethoxyethyl) phosphorodithioate
paraoxon	O,O-diethyl O-p-nitrophenyl phosphate
parathion	,, ,, ,, phosphorothionate
diazoxon	,, ,, O-(2 *iso*propyl-4-methyl-6-pyrimidinyl) phosphate
diazinon	,, ,, O-(2 *iso*propyl-4-methyl-6-pyrimidinyl) phosphorothionate
ethyl DDVP	O,O-diethyl O-2.2 dichlorovinyl phosphate
propyl paraoxon*	O,O-di-n-propyl O-p-nitrophenyl phosphate
propyl parathion*	,, ,, ,, phosphorothionate
*iso*propyl paraoxon*	O,O-di-*iso*propyl O-p-nitrophenyl phosphate
butyl paraoxon*	O,O-di-n-butyl O-p-nitrophenyl phosphate

Bimolecular rate constants of the reaction of the oxygen-compounds with the cholinesterase and ali-esterase were measured (Table I) as described before (VAN ASPEREN & OPPENOORTH, 1960).

TABLE I

Bimolecular rate constants of the inhibitory reactions at 27° C (Litres Moles^{-1} Min.$^{-1}$)

	Cholinesterase (Substrate acetylcholine 1.5×10^{-2} M)	Ali-esterase (Substrate phenylbutyrate emulsion)
methyl paraoxon	0.2×10^{6}	15×10^{6}
DDVP	8×10^{6}	10×10^{6}
chlorooxon	5×10^{6}	60×10^{6}
malaoxon	2.5×10^{6}	7×10^{6}
paraoxon	5×10^{6}	80×10^{6}
diazoxon	40×10^{6}	40×10^{6}
ethyl DDVP	15×10^{6}	50×10^{6}
propyl paraoxon	0.1×10^{6}	100×10^{6}

Estimation of susceptibility

Susceptibility was measured in the same way as described earlier (OPPENOORTH, 1959a). For most of the toxicants a contact method was employed, in which the insecticides in 2 μl of peanut oil were applied to the walls of small jars. Twenty flies, males and females mixed, were kept in these jars for 24 hours at 25°, after which mortality was estimated. Malathion was topically applied because with the highly resistant strains the contact method failed to give sufficient mortality. With an Agla micrometer syringe 0.5 μl of a solution in olive oil was applied to the dorsal thorax. In some of the experiments with synergists mixtures of the

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*) Samples of propyl- and *iso*propyl paraoxon were kindly supplied by Agrochemie N.V., Arnhem, Netherlands; those of propylparathion and butylparaoxon by Shell, Woodstock Agricultural Research Centre, Sittingbourne, England.

products were topically applied in 1 μl of acetone by means of an Agla micrometer syringe.

Rearing and selection

Flies were reared on a milkpowder-yeast diet (OPPENOORTH, 1959a). Resistant flies were kept under slight selection pressure in order to maintain the original properties of the strains. However there are indications that in some strains the resistance has increased somewhat over a period of several years. In some of the resistant strains which were obtained by special crosses, resistance is believed to be completely due to a gene which also causes low ali-esterase activity. These strains were not exposed to insecticides but a periodic estimation of ali-esterase activity was carried out to detect possible contamination.

Strains of flies

A susceptible and a number of organophosphate-resistant strains have been used. Of most of these strains detailed information on their resistance to a number of organophosphates has already been published (BUSVINE, 1959; OPPENOORTH, 1959a). Origin and main characteristics of the strains are listed in Table II.

The breeding of strain Ka

In a previous paper (OPPENOORTH, 1959b) the derivation of a strain Fa from strain F and S has been described. In this strain the gene *a* for low ali-esterase activity of strain F has been combined with the genome of strain S. A similar procedure was followed in order to obtain a strain Ka in which the *a* gene of strain K is introduced into the genome of strain S.

The method of measuring ali-esterase activity of individual flies was the same as employed previously. According to the activity found with the Warburg technique it was possible to classify the flies into aa, aa^+ and a^+a^+ genotypes. An f_1 and first backcross with S were obtained from mass crosses. In the rest of the crosses single pairs were used. In the backcrosses aa^+ and a^+a^+ flies were obtained and a number of females were paired with S males. After they had produced a batch of eggs, the females were tested in the Warburg, and the offspring of an aa^+ female was selected for the next cross. After three backcrosses it was attempted to breed a pure aa strain by inbreeding. The number of aa flies obtained in these crosses was always lower than expected, but a pure aa strain was acquired after five generations of inbreeding.

As a result of the three backcrosses the percentage of genetic material derived from strain K will be $1/2^4 = 6.25\%$ if the effect of linkage is neglected. The actual percentage will be higher as a result of linkage, but cannot be calculated unless the map length of the total genetic material and of the chromosome on which gene *a* is located is known. The average genetical length linked with the gene that is introduced was shown by BARTLETT & HALDANE (1935) to be $\frac{1-2^{-n}}{n}$ Morgan Units on both sides of the gene, where n is the number of backcrosses. Since in housefly males crossing over is negligible (MILANI & TRAVAGLINO, 1957; HIROYOSHI, 1960) females were used for backcrossing with strain

TABLE II *Characteristics of the strains*

Strain	degree of resistance $\left(\frac{\text{LD}_{50}\text{ res. strain}}{\text{LD}_{50}\text{ susc. strain}}\right)$ parathion contact	diazinon contact	malathion topically applied	Genetic basis of resistance	Breakdown capacity mμg/fly /2 hr (see Table III) diazoxon	malaoxon	Origin, selection and previous name
S	—	—	—	—	5*	4*	Susceptible strain, kept for many years in the laboratory.
D	10	7	3	mainly caused by *a* allele	15	—	Denmark. Collected after the use of parathion. Selected with parathion (KEIDING 1956, strain 79b).
Cooper	—	40	—	*a* allele present, probably also other factors	14	—	Selected in the laboratory with diazinon. Kindly put at our disposal by Mr. Wickham of Cooper Technical Bureau Ltd., Berkhamsted, Herts, England.
F	25	52	9	caused by *a* allele and other factors	25	—	Denmark. Collected after the use of diazinon. Selected with diazinon (KEIDING 1956, strain 203a).
Fa	9	8	—	caused by *a* allele only	17	—	Obtained by introducing the *a* allele of strain F into strain S (OPPENOORTH 1959b).
Fb	2	8	—	one or more genes, not an *a* allele	5*	—	Obtained from crosses of strain F and S (OPPENOORTH 1959b).
Fc	5	30	—		—	—	
C	32	35—60†	4	mainly caused by *a* allele	76	29	Italy. Collected after the use of diazinon. Selected with diazinon (SACCA 1957)
K	—	100	8	caused by *a* allele and other factors	75	22	U.S.A. N. Jersey. Collected after the use of diazinon. Selected with diazinon (HANSENS 1958, Rutgers strain).
Ka	—	40	4	caused by *a* allele only	55	—	Obtained by introducing the *a* allele of strain K into strain S.
H	low according to BUSVINE (1959)		35	mainly caused by *a* allele	—	11	U.S.A. Georgia. Collected after the use of malathion. Selected with malathion (SCHOOF & KILPATRICK 1958, Anderson 45 strain).
G	3	2	> 40	mainly caused by *a* allele	7*	118	Same as strain H FAY *et al*, 1958, Bethesda 45 strain).

S. In these females crossing over will occur and therefore parts of the chromosome on which gene *a* is located can be exchanged. If males had been chosen the chromosome would have been selected as a whole and transported to strain Ka. The *aa* strain obtained showed a reduced viability as compared to the other strains. Since this was thought to be the result of inbreeding, and not an effect of gene *a* as such, another cross with strain S was carried out. By inbreeding a pure *aa* strain was produced with a much better viability. This strain has been called Ka.

The breeding of strain Fc

The isolation from strain F of a resistant substrain, Fb, that does not contain gene *a* has been described before. Since strain Fb was lost by accident it was attempted to breed another strain from strain F that would lack allele *a* but would carry other genes for resistance. To this end an f_1 of strain S and F was backcrossed to strain S. From the offspring of this backcross single pairs were isolated, and after these had produced eggs the ali-esterase activity of the parent flies was determined. Three pairs were found to be of a^+a^+ constitution and their offspring were combined and selected with diazinon. By further selection with diazinon for some generations a fairly resistant strain, Fc, was obtained with high ali-esterase activity. This strain had a higher resistance than strain Fb, (see Table II), which proves that more or more effective resistance factors derived from strain F are present in Fc than in Fb.

Estimation of detoxication-capacity of homogenates

Two procedures were followed to determine the breakdown capacities of homogenates for the oxygen analogues of the organophosphate insecticides.

A. *Measurement of the maximum amount of organophosphate that can be completely degraded in a certain period of time.*

To a series of samples of a homogenate increasing amounts of the toxicants were added. After incubation for some hours the presence of inhibitor was tested by adding a small amount of a test enzyme and measuring the resulting inhibition. The flies were homogenized in ice cold 1% NaCl solution in a cooled Waring blendor for ½ min. Homogenates of six flies per ml were incubated with the toxicant at 27° C under vigorous shaking. After 2 or 4 hours 1 ml portions of the samples were transferred to Warburg flasks, and 0.8 ml of a homogenate of three fly heads per ml was added. The final medium contained 3% NaCl and 0.025 M $NaHCO_3$, and was gassed with 5% CO_2 in N_2. After incubation for 30 minutes at 37° C, acetyl choline was tipped in (final concentration 1.5×10^{-2} M) and cholinesterase activity measured. In some experiments ali-esterase was used as a test enzyme. In this case the hydrolytic activity was estimated at 27° C with methylbutyrate emulsion as a substrate and 1 ml of a homogenate containing one fly per ml as source of ali-esterase. The amount of toxicant required for inhibition of the test enzyme is very small as compared to the amounts added to the breakdown enzyme. Only 2 $\mu\mu$ moles of diazoxon and paraoxon and 4 $\mu\mu$ moles of chlorooxon and malaoxon are necessary to obtain 50% inhibition of the test enzyme.

B. *Measurement of the amount of organophosphate remaining after incubation with a homogenate for a certain period of time.*

The flies were homogenized and incubated as in method A. For the biochemical estimation of the remaining organophosphate different dilutions of the incubated mixture were prepared and added to samples of test enzyme in Warburg flasks. Different dilutions were prepared in order to obtain a dose-inhibition curve of the unknown amount that could be compared with a standard dose-inhibition curve. If the two curves could not be reasonably fitted the experiment was discarded. Conditions of manometric assay were the same as described for method A. The method is very sensitive but relatively inaccurate.

RESULTS

Breakdown capacity of homogenates of different strains

Table IV shows the amounts of organophosphate broken down during an incubation period of 2 and 4 hours (method A). As shown before (VAN ASPEREN & OPPENOORTH, 1960) the rate at which paraoxon and diazoxon are eliminated is practically independent of substrate concentration. This was borne out by the fact that the test enzyme generally is either inhibited completely, or not at all. An example of this type of reaction is shown in Table III (strain C with diethyl DDVP). With malaoxon, chlorooxon, DDVP and methylparaoxon a partial inhibition of the test enzyme is frequently found, which is to be expected if the rate of breakdown appreciably decreases at low concentrations (Table III, strain G with malaoxon).

In the first case (degradation of diethyl compounds by strain D, F, Fa, C, K, Ka) it must be concluded that the breakdown enzymes have a high affinity for the phosphates, and that the values of Table IV represent the maximum rate of breakdown at full saturation of the enzyme. This was also confirmed by some experiments with method B. In the second case (degradation of malaoxon and some other dimethyl compounds by strain C and G) the values found (Table IV) do not represent the maximum breakdown capacity, but are lower. It will be apparent that the meaning of the breakdown values is less clear in this case. Nevertheless they are presented here since they constitute the only information available about breakdown at low concentrations which may be of special importance for resistance.

Breakdown of diazoxon, paraoxon and ethyl DDVP

It is apparent from Table IV that in many experiments the amounts eliminated in 4 hours are less than twice those eliminated in 2 hours. This is probably caused by a gradual denaturation of the breakdown enzyme, for which evidence was also found in other experiments. Therefore the 2 hours values will be the best measures of the breakdown capacities of the strains. Three levels of elimination are found in different strains.

1). In strains S, G, Fb and Fc only a small elimination is found that does not increase with time, and is probably caused by a binding to proteins of the homo-

TABLE III

Estimation of the maximum amount of organophosphate that can be "completely" degraded in a certain period of time (Method A)

	Breakdown period 2 hours						Breakdown period 4 hours					
Amount of diethyl DDVP added to homogenates of strain C ($\mu\mu$M/fly)	0	180	240	320	400	600	320	400	600	800	1000	
Activity of test enzyme in the Warburg: μl CO_2 production in 30 min.	134	129	118	3	0	3	133	133	6	3	6	
Amount of malaoxon added to homogenates of strain G ($\mu\mu$M/fly)	0	170	330	500	670		330	500	670	830	1000	1160
Activity of test enzyme in the Warburg: μl CO_2 production in 30 min.	124	146	110	35	14		141	134	110	45	27	11

Homogenates of 6 flies per ml are incubated for 2 or 4 h with the indicated amounts of OP-compound. The test enzyme is then added to detect remaining inhibitor. The dotted line is drawn where 50% inhibition is obtained. In Table IV the amounts on both sides of these lines, resulting in more and less than 50% inhibition of the test enzyme, are listed.

TABLE IV

Amounts of organophosphates that can be "completely" eliminated by homogenates of flies of different strains in the indicated periods of time (Method A). Explanation see Table III

	diazoxon		paraoxon		ethyl DDVP		malaoxon		chlorooxon		DDVP	methyl paraoxon
	2 h	4 h	2 h	4 h	2 h	4 h	2 h	4 h	2 h	4 h	2 h	2 h
S	10—20	15—20	10—15	10—15	10—20	10—20	8—17		10—20	10—20	15—20	5—10
	15—20	15—20					8—13	8—13	10—20			10—20
D	52—63	125—167	40—60	80—100							10—20	20—30
	42—50	75—104										
Cooper	40—60											
F	67—83	104—125	33—50	75—125	40—80							
	83—104	104—160	40—60	80—100								
	83—104	125—146										
Fa	62—83	62—104	42—58	58—75								
	42—63	83—104										
	42—63	83—104										
Fb	15—20	15—20										
C	210—250	420—520	310—320	520—630	240—320	400—600	83—108		200—250	350—400	80—120	80—160
	250—330	420—520	210—290	500—510			83—108		200—250	300—310		
	250—300						42—83					
K	250—330	250—420	210—220	330—420			33—80					
	210—250	420—530					62—83					
							58—83	146—210				
Ka	180—220											80—160
	180—220											
	150—200											
H							21—42					
							21—42					
G	20—25	20—25			20—40		330—500	660—830	10—40		10—20	10—20
							330—500		40—80			
							167—250		20—40	< 80		

genate. Of these strains S and G are susceptible to these organophosphates whereas strains Fb and Fc are resistant, but do not contain the *a* gene.

2). In the strains D, F, Fa and Cooper an enzyme is present with a moderate capacity. Since strain Fa only differs from strain S in possessing gene *a* the breakdown capacity found must be responsible for all of the resistance. This probably also applies to strain D, which shows the same breakdown capacity and resistance. Strain F is much more resistant due to the presence of more than one factor (OPPENOORTH, 1959b). In the Cooper strain resistance is also of a high level, and it seems likely that here too other factors occur. It can be concluded that these strains have a breakdown enzyme with a moderate capacity which is related to the ali-esterase and produced under the influence of a mutant gene *a*. Whether the *a* alleles present in these strains are identical is not known.

3). The strains C, K and Ka contain an enzyme with a much higher capacity. In strain Ka, just as in Fa, the breakdown capacity due to gene *a* must be responsible for the resistance. Thus the presence of at least two different alleles of gene *a* causing different levels of diazinon-parathion resistance is clearly demonstrated. There are indications (see below) that these alleles produce two qualitatively different breakdown enzymes, and not merely a different amount of the same enzyme.

The breakdown capacity found in strains K and C is somewhat higher than in strain Ka. This may be caused by the influence of secondary factors in these strains that enhance the production or the activity of the enzyme present which have been removed by the backcrossings. It seems unlikely that the clear cut difference in resistance between K and Ka is due to the difference in breakdown activity. It is concluded, therefore, that in strain K gene *a* is an important, but not the only gene for resistance.

For each of the enzymes mentioned equal rates of breakdown were observed for the different ethyl phosphates tested. The significance of this will be discussed under "reaction mechanism".

A good parallelism between degree of resistance to diazinon and parathion and breakdown capacity is found in the strains in which resistance depends largely on gene *a*. Strains C and Ka show high levels of resistance and of breakdown, in strains D and Fa both levels are much lower. In strains S and G, without resistance to these compounds, no breakdown activity occurs. The strains F, Fb, Fc and K in which other resistance factors are present show no such parallelism, indicating that the other genes for resistance in these strains do not lead to production of breakdown mechanisms that show up under the conditions of our *in vitro* experiments.

Breakdown of malaoxon and other methyl compounds

The breakdown of malaoxon has been measured by methods A and B (Tables IV and V).

In strain S only about 10 $\mu\mu$ moles of malaoxon per fly is removed, and the amount does not increase when the incubation period is prolonged. Also at higher concentrations (method B) no significant breakdown can be detected.

In strain H only a low breakdown is found with both methods (30 $\mu\mu$ moles in 2 h in method A, 40 $\mu\mu$ moles in 1 h in method B).

In strains C and K a much higher elimination is found. With method A this is about 80 $\mu\mu$ moles in 2 h; with method B at the highest concentration tested it is 220 $\mu\mu$ moles in 1 h. This indicates a strong dependence on concentration which is confirmed by the results shown in Table V (Km value probably in the order of 10^{-6} M).

TABLE V

Breakdown of malaoxon by homogenates of houseflies of different strains (method B). Explanation in text.

Strain	Amount added per fly ($\mu\mu$moles)	Number of estimations	Estimated amount in $\mu\mu$moles after			Concentration during breakdown period M x 10^7	Amount broken down per fly per hour ($\mu\mu$moles)	
			1 h	2 h	3 h		during 1st h.	during 2nd h
S	17	1	5	4		1—0.3	12	1
	200	1	220			12	—20	
	400	1			370	24—22	—	
C	150	3	84			9—5	66	
	275	3	116	27		17—2	159	89
	550	3	330	255		33—15	220	75
K	200	2	137			12—8	63	
Ka	200	2	130			12—8	70	
H	400	3	360			24—22	40	
G	300	4	34			18—2	266	
	400	10	140			24—9	260	
	625	3	285			38—17	340	
	1250	3	870	640		75—38	380	230

In strain G a very high elimination rate is found, about 400 $\mu\mu$ moles in 2 h with method A, and up to 380 $\mu\mu$ moles in 1 h with method B. Although the concentration effect is also apparent here, it is smaller than in the reaction of strain C which indicates that the Km value of the reaction is lower.

When we try to correlate breakdown capacity with degree of resistance to malathion, it becomes at once apparent that the breakdown found in strain H is much too low to account for its resistance. There is strong evidence, however, that resistance in this strain is brought about by a breakdown enzyme of similar nature as in strain G (see below). This enzyme is probably inactivated in some way in the homogenates. The difference in resistance of the remaining strains C, K and G is in accordance with the breakdown at lower concentrations (method A), but not with that at higher concentrations where only a small difference between strains C and G is found. Since it is known from genetical experiments that ali-esterase derived enzymes are the main resistance factors in strains C, K and G, the conclusion seems warranted that the degree of resistance is mainly determined by the breakdown-capacity at low concentrations.

Breakdown of DDVP and methylparaoxon is low and only a low order of resistance is present to these compounds.

Chlorooxon is rapidly degraded by homogenates of strain C. Yet, when topically applied, only a five fold resistance is observed. According to Busvine (1959) a similar factor is found with chlorthion. It cannot yet be explained why the breakdown mechanism does not cause a higher degree of resistance to this compound. In strain G there is only very slow breakdown, but the resistance to chlorooxon is higher (10 ×). Probably, therefore, this resistance is brought about by other factors.

It can be concluded that there is evidence for 4 different types of breakdown enzymes present in different strains:

1) an enzyme in strains F, Fa, D and Cooper, with a high affinity for paraoxon and diazoxon ($Km \leqq 10^{-8}$ M). Assuming that the enzyme is present in the same amount as the ali-esterase in S, it can be calculated that its turnover number (t.o.n.) is 0.05 per minute.

2) an enzyme in strains C, K and Ka with a high affinity for paraoxon and diazoxon, ($Km \leqq 10^{-8}$ M), with a t.o.n. of 0.25 per minute. The enzyme can also degrade some methyl compounds ($Km \sim 10^{-6}$ M), with a t.o.n. of about 0.4.

3) an enzyme in strain G, which can degrade malaoxon with a t.o.n. of 0.7.

4) an enzyme in strain H, which is inactive in our *in vitro* experiments, but must be capable of degrading malaoxon *in vivo*.

Reaction mechanism

It is generally held that the reaction of esterases with organophosphates can be represented as follows (Dixon & Webb, 1958).

$$\begin{array}{lcl} EH + PX & \leftrightarrows & EH\text{-}PX \\ EH\text{-}PX & \rightarrow & EP + HX \\ EP + H_2O & \rightarrow & EH + POH \end{array}$$

where EH stands for the enzyme, PX for the organophosphate, P for the (alkyl-O)$_2$-PO-group that phosphorylates the enzyme, and HX for some acidic or phenolic compound. The ali-esterase of susceptible flies is irreversibly phosphorylated which means that the rate of the third step of the reaction is practically nil. Since the breakdown enzymes are related to the ali-esterase their reaction mechanism is probably similar, the main difference being in the rate of dephosphorylation. It has been mentioned that the rate of breakdown of different diethyl organophosphates, for which the enzymes have a high affinity, is the same. This should be expected if the third reaction is the rate limiting step, since these compounds will all produce the same phosphorylated enzyme.

Results on the joint action of different organophosphates, described below, reinforce the conclusion that the reaction mechanism of the breakdown enzymes is similar to that of other esterases. Therefore the breakdown is thought to consist of a hydrolysis of the organophosphates to the dialkylphosphate moiety and an acidic or phenolic compound. Experiments to check this by estimating the breakdown products of P^{32}-labelled organophosphates are under way.

Inference on the properties of the breakdown enzymes from the in vitro *inhibition of cholinesterase*

When homogenates of susceptible flies are incubated with organophosphates and inhibition of cholinesterase is plotted against amount of toxicant, only little inhibition is observed at low inhibitor concentrations. This has been shown to be mainly due to the presence of the ali-esterase which reacts more rapidly with the inhibitor than the cholinesterase (ChE) and thus removes a considerable part of it. The rates at which different organophosphates react with the ali-esterase are thus reflected in the ChE-inhibition curves (VAN ASPEREN & OPPENOORTH, 1960).

Similar experiments on cholinesterase inhibition were used for comparing the rates at which ali-esterase and breakdown enzymes react with a given organophosphate. These experiments were performed at 27° C to avoid denaturation of the breakdown enzymes, which proved rather labile[1]).

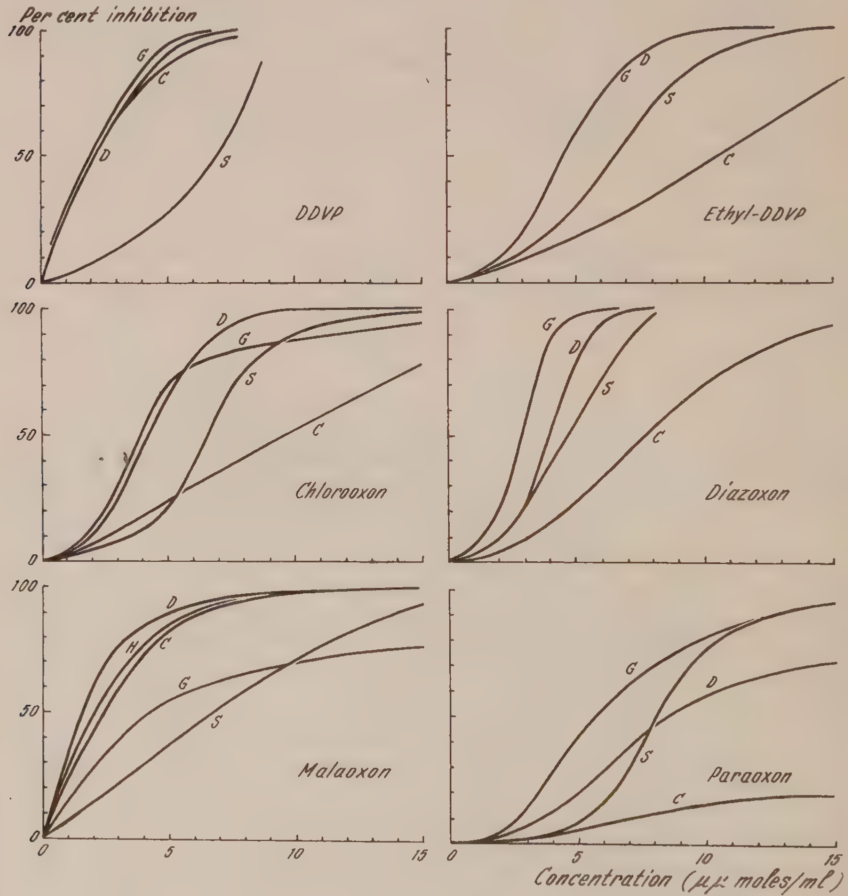

Fig. 1. Concentration inhibition curves for cholinesterase and six OP-compounds.

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[1]) In a previous paper experiments were described that were carried out at 37° C (VAN ASPEREN & OPPENOORTH (1960, Fig. 1, 2 and 3). The results of these experiments are likely to be affected by denaturation.

Some introductory remarks may help in interpreting the results, which are shown in Fig. 1. The rate v at which the ali-esterase or breakdown enzyme reacts with an organophosphate (expressed as the fraction of the latter that is converted per unit of time) is determined by two factors: 1) the concentration of free enzyme [Ef] and 2) the rate constant k of its phosphorylation: $v = k. [Ef]$. Free enzyme concentration is dependent on total enzyme concentration and it varies with time. If no dephosphorylation occurs one molecule of free enzyme will be lost for every organophosphate molecule that is bound and free enzyme concentration will steadily decrease. Any dephosphorylation such as occurs in the reaction between breakdown enzymes and their organophosphate substrates tends to increase the concentration of free enzyme.

If compared with the curve for strain S three different types of curves can be distinguished.

1) curves that lie more to the left, indicating that the breakdown enzyme reacts more slowly with the OP-compound than the ali-esterase.

2) curves, that lie more to the right. This means that the breakdown enzyme is a more efficient competitor of ChE for the OP-compound than the ali-esterase. Possible explanations for this behaviour are: (i) the breakdown enzyme is present in a higher concentration than the ali-esterase, (ii) its phosphorylation constant k is higher, and (iii) free enzyme concentration is higher as a consequence of dephosphorylation. The latter factor will be much more important at high OP-concentrations.

3) an intermediate type, in which at low concentrations the curve lies more to the left, due to a lower reaction rate, whereas at high concentrations it lies more to the right, due to the ability of the breakdown enzyme to recover after the reaction with the organophosphate.

We shall now see how these data compare with those on breakdown as shown in Tables IV and V.

The curves of *strain D* always lie to the left of those of strain S, except for paraoxon at higher concentrations. Either the breakdown enzyme of strain D is present in smaller amounts than the ali-esterase in strain S or it is less readily phosphorylated. The intersection of the curves in the case of paraoxon indicates the ability of the breakdown enzyme to degrade paraoxon which is in full agreement with the data in Table IV. The ability to degrade diazoxon probably does not show up because of the very rapid inhibition of cholinesterase by this compound (see Table I), which leaves little opportunity to the breakdown enzyme to affect this process.

The curves of *strain C* lie more to the right than those of strain S for the diethyl compounds, both at the lower and the higher concentrations. Since there is evidence that the breakdown enzyme in strain C is present in about the same concentration as the ali-esterase in strain S, the most plausible conclusion is that the phosphorylation rate constants are higher for the breakdown enzyme than for the ali-esterase. It can further be concluded that the diethyl compounds studied are degraded at a reasonable speed, even at the relatively low concentrations used in these experiments. This agrees quite well with the data on breakdown. The data on breakdown of the dimethyl compounds (Table IV) show that chlorooxon is much more rapidly degraded than DDVP and malaoxon. Since dephosphorylation must be equally rapid for all three compounds it must be assumed that the difference is in the rate of phosphorylation. Table I shows that the phosphorylation rate constant of the ali-esterase is much higher for chlorooxon (6×10^7) than for DDVP (10^7) and malaoxon (7×10^6). From the relative positions of the S- and C-curves in Fig. 1 it can be concluded that the rate constants of the breakdown enzyme must be much lower than those of the ali-esterase for DDVP and malaoxon (C-curve left of S-curve) and about equal for chlorooxon (little difference between S- and C-curves at low concentrations of chlorooxon). There can be little doubt, therefore, that phosphorylation of the breakdown enzyme by chlorooxon proceeds much faster than by DDVP or malaoxon, which is in agreement with the assumption made above.

The curves of *strain G* always lie to the left of those of strain S at low organophosphate concentrations. Two causal factors can be important: 1) the concentration of the breakdown enzyme may be lower than that of the ali-esterase in strain S. Some evidence for this is presented on page 327, and 2) the phosphorylation rate constants of the breakdown enzyme may be lower than those of the ali-esterase. The intersection between the G- and S-curves in the case of malaoxon clearly indicates the ability of the breakdown enzyme in strain G to degrade malaoxon. According to Fig. 1 the breakdown enzyme is very slowly phosphorylated by DDVP which may explain its inability to degrade this compound. The data are not conclusive in explaining the intermediate position of chlorooxon between malaoxon and DDVP in Table IV.

On page 328 evidence is presented for the occurrence in *strain H* of a breakdown enzyme capable of degrading malaoxon *in vivo*. The position of the curve for strain H in Fig. 1 provides no evidence for a reaction of such an enzyme with malaoxon under our *in vitro* conditions, which agrees with the results on breakdown given in Tables IV and V.

Esterase activity of breakdown enzymes

In organophosphate resistant flies ali-esterase activity with methylbutyrate as a substrate is about 20% of that of susceptible flies. With a number of other substrates the difference is smaller or absent. The question arises whether the breakdown enzymes take part in the remaining esterase activity to some of these carboxyesters, or whether this is completely due to other enzymes, which are not different in the strains.

The possible carboxyesteratic activity of the breakdown enzymes was studied in the following way. A series of samples of a homogenate was incubated with increasing doses of an organophosphate. Concentrations were chosen in such a way that at the end of the incubation period the phosphate had been completely removed in a number of samples, but only partially in others. The samples were then transferred to Warburg vessels and a carboxyester was added. In Fig. 2 the relation between methylbutyrate hydrolysis and amount of organophosphate added is shown for homogenates of strains D and C. Beyond the highest concentration which is known to be broken down by the strain during the incubation period hydrolytic activity falls rather sharply from a higher to a lower level. The lower level apparently represents the activity of organophosphate insensitive enzymes. The higher level found in samples in which the organophosphate has been broken down during the incubation period is probably caused by the activity of these enzymes plus that of the breakdown enzyme which is no longer occupied by the phosphate. Therefore this enzyme would have an activity of 5 and 2 μl CO_2 (per fly per 30 minutes) in strains D and C respectively with methylbutyrate as a substrate which is only 1 or 2% of the activity of the ali-esterase of susceptible flies.

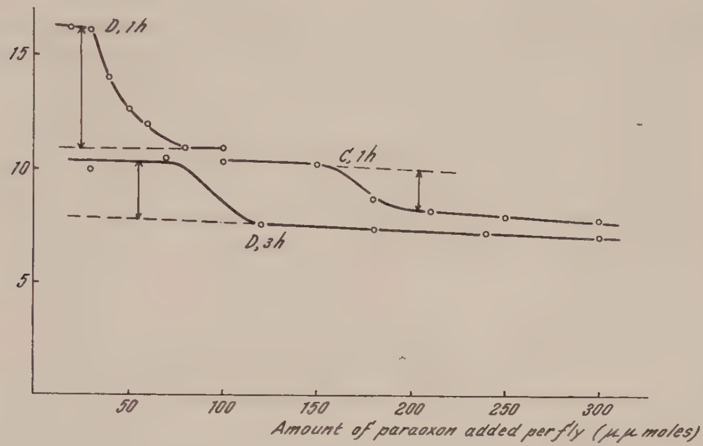

Fig. 2. Hydrolysis of methylbutyrate after incubation with paraoxon in homogenates of flies of strains D and C. Homogenates of six flies per ml were incubated for 1 or 3 h with the indicated amounts of paraoxon, after which the hydrolytic activity was measured. Possible activity of the breakdown enzymes indicated by arrows. Ordinate: μl CO_2/30 min./fly.

Similar curves have been obtained with tributyrin and phenylbutyrate. No indication was found of activity of the breakdown enzymes towards triacetin and phenylacetate.

Carboxyesteratic activity is higher in strain D than in strain C. The reverse is true for the breakdown of the inhibitors which is much higher in strain C. It can be concluded therefore that the properties of the two enzymes are different.

It should be pointed out that an alternative explanation of the results of Fig. 2 cannot be excluded. The activity that was ascribed to the breakdown enzyme could have been brought about by another enzyme in the homogenate that would be reversibly inhibited. Such an enzyme should have a very high affinity for the inhibitor.

Inhibition of breakdown enzymes

The ali-esterase which is formed in susceptible flies under the influence of gene a^+ is readily inhibited by a large number of organophosphates. The altered ali-esterases can hydrolyse some of these toxicants, but not all of them. There are two possible explanations for the fact that an inhibitor of the ali-esterase is not split by the breakdown enzyme: either the breakdown enzyme is not phosphorylated or it is phosphorylated but, just as is the case with the ali-esterase, no significant dephosphorylation takes place. If there were organophosphates that could phosphorylate the enzyme irreversibly, they would be expected to block the breakdown enzymes.

Since it was found that the breakdown enzyme of strain G can split malaoxon but does not attack diethyl phosphates, it was investigated whether these would phosphorylate the enzyme. A homogenate of flies of strain G was divided into two samples. One of them was preincubated with an amount of paraoxon of 10 $\mu\mu$ moles per fly, the other was not. To both samples 300$\mu\mu$ moles of malaoxon were added, and the amount of malaoxon remaining after one hour of incubation was measured biochemically (method B). The results are shown in Table VI. Whereas malaoxon had been broken down nearly completely in the sample without paraoxon, only a slight elimination had taken place in that preincubated with paraoxon. It can be concluded that the breakdown enzyme of strain G resembles the original ali-esterase in that it is phosphorylated by this diethyl compound.

TABLE VI

Inhibition of breakdown enzymes (explanation in text).

Strain	Nature and amount of inhibitor ($\mu\mu$ moles/fly)		Duration of preincubation period (h)	Nature and amount of substrate added ($\mu\mu$ moles)		Amount broken down per fly in one hour ($\mu\mu$ moles)	
						with inhibitor	without inhibitor
G	paraoxon	10	1	malaoxon	300	51	284*
	,,	10	0	,,	300	51	249
	propyl paraoxon	12	0.5	malaoxon	300	—30	245*
C	propyl paraoxon	12	1	malaoxon	150	—10	51†
	,, ,,	12	0.2	,,	150	11	69†
	propyl paraoxon	10	1	diazoxon	120	2	120*
	,, ,,	12	0.5	,,	120	—8	62†
	,, ,,	12	0	,,	120	39	109
	*iso*propyl paraox.	12	0.5	diazoxon	120	—20	82†
	,, ,, ,,	12	0	,,	120	36	85
	propyl paraoxon	10	0.5	diazoxon	120	—20	(52 in 2.5 h)
D	propyl paraoxon	12	0.5	diazoxon	40	9	33†
	,, ,,	12	0	,,	40	9	23

* control not preincubated.

† control preincubated, and perhaps partly denatured during this procedure. Negative values mean that the estimated amount is higher than the amount added.

The breakdown enzyme present in strain C is known to degrade both dimethyl and diethyl compounds and in this case it was tested whether perhaps dipropylphosphates would irreversibly phosphorylate this enzyme. Breakdown of malaoxon or diazoxon was measured in samples of homogenates with and without previous incubation with n-propyl- or *iso*propyl-paraoxon. The results are shown in Table VI. Amounts of 10—12 $\mu\mu$ moles per fly significantly inhibited breakdown of malaoxon or diazoxon. This was also the case in strains G and D. Therefore propylparaoxon can phosphorylate the three different breakdown enzymes present in strains D, C and G and can thus inhibit *in vitro* breakdown of those phosphates to which the strains are resistant. In some experiments inhibitor and diazoxon were added simultaneously to the homogenate (preincubation period 0 h). Although there is some breakdown of diazoxon in these cases it is still significantly lower than in the control without inhibitor. It follows that even in competition with diazoxon the inhibitor can phosphorylate the breakdown enzyme. This points to a high rate constant of the inhibitory reaction. It should be pointed out that the rate constant of propyl-paraoxon with the ali-esterase of susceptible flies is the highest listed in Table I.

It was attempted to find out whether phosphorylation by propyl-paraoxon was completely irreversible. In one experiment a homogenate of strain C was incubated for half an hour with 10 $\mu\mu$ moles of inhibitor per fly, and breakdown of diazoxon was measured after 1 and 2.5 hours. No breakdown was found after 1 hour, but an appreciable amount had been eliminated after 2.5 h. This might indicate that a slow dephosphorylation occurs. This point needs further investigation in view of its importance for possible use of breakdown inhibitors as synergists.

Amount of breakdown enzymes

In a previous communication (VAN ASPEREN & OPPENOORTH, 1960) it has been calculated that the amount of the ali-esterase made under the influence of gene a^+ is between 7 and 12 $\mu\mu$ moles per fly. The upper limit has been derived from OP-inhibition values found for the ali-esterase in S-homogenates. The reaction of the breakdown enzymes with propyl-paraoxon in a similar way enables the calculation of an upper limit of their amount. To this end the inhibition of breakdown was measured which was caused by preincubation with small amounts of propyl-paraoxon. Since the breakdown enzyme seems to have a high affinity for the inhibitor, it is thought that the amount of inhibitor added will be largely used up in the reaction with this enzyme, and it is expected, therefore, that the upper limit calculated will not be much higher than the actual amount. In homogenates of strain C inhibition of breakdown of diazoxon was measured using method A, and in homogenates of strain G inhibition of breakdown of malaoxon using method B. In every experiment the breakdown in a sample of homogenate that was preincubated with inhibitor was compared with that of another sample without inhibitor. The upper limit found for the amount of breakdown enzyme in strain C is 12 $\mu\mu$ moles, that for strain G is about 6 $\mu\mu$ moles per fly. It should be borne in mind, however, that the method used is rather inaccurate, and it seems wise to conclude only that the values found are of the same order of magnitude as those found for the ali-esterase.

Inhibition of the breakdown enzymes in vivo*: synergism*

The very effective inhibition *in vitro* of the breakdown enzymes that have been shown to be mainly responsible for resistance suggests that it might be possible to eliminate the resistance if these enzymes could also be inhibited *in vivo*. In this study n-propyl-paraoxon was chosen since it has a relatively low toxicity, probably as a result of its low reaction rate with the cholinesterase (Table I).

In Table VII results are shown of topical application in acetone of mixtures of this compound with *malathion* to the susceptible and to two malathion resistant strains. A very distinct synergism was obtained with strains G and H, with doses of propyl-paraoxon that caused a mortality of only 0—10% when applied alone. Also with strain S a synergistic action was found. This can probably be explained by the fact that propylparaoxon inhibits the ali-esterase and possibly other enzymes

TABLE VII

Synergism of propyl paraoxon and malathion in a susceptible strain (S) and two malathion resistant strains (G and H)

Percentage mortality 24 h after the topical application of 1 μl of a solution in acetone

μg per fly of prop. paraox. / malathion	S		G				H			
	0	0.1	0	0.1	0.2	0.4	0	0.05	0.1	0.2
0	—	7	—	0	0	10	—	5	45	50
0.035	—	—	—	—	—	—	—	20	75	90
0.07	—	33	—	—	5	0	—	30	90	95
0.15	0	90	—	—	15	30	—	30	95	100
0.31	3	100	—	20	55	35	—	75	100	100
0.62	100	—	—	30	60	75	—	100	100	100
1.25	100	—	—	80	90	100	—	—	—	—
2.5	—	—	0	95	—	100	0	—	—	—
5	—	—	0	95	100	100	0	—	—	—
10	—	—	0	100	—	—	0	—	—	—
20	—	—	0	100	—	—	0	—	—	—

which normally would react with the malaoxon formed. The LD_{50} of this strain is lowered by a factor of five. In the resistant strains a much stronger synergistic effect is found. Here the LD_{50} is lowered by a factor of more than 50 and 100 in strains G and H respectively. This very clear *in vivo* effect of an inhibitor of the breakdown enzyme strongly supports the view that the activity of this enzyme constitutes the main resistance mechanism of the strains.

Resistance of strains G and H can be largely overcome by addition of small amounts of propyl-paraoxon, but not completely. This may be explained by an incomplete block of the breakdown enzymes, or by the presence of other resistance factors. Some resistance to propyl-paraoxon is found in strain G, probably also due to other resistance factors. Strain H was found to be slightly more susceptible to propyl-paraoxon than strain S.

It must be remembered that only a very low breakdown of malaoxon was found in homogenates of strain H. Since resistance in this strain is known to be monogenic and low ali-esterase activity is present, it is practically certain that resistance is brought about by an altered ali-esterase. It seemed likely therefore that the low *in vitro* breakdown activity has to be ascribed to some kind of inactivation of the enzyme. The distinct synergistic effect of a small amount of propyl-paraoxon strongly supports this view.

Results of topical applications in acetone of mixtures of propyl-paraoxon and *diazinon* are shown in Table VIII. In the susceptible strain S and in strain Fc with genetical constitution a^+a^+ the LD_{50} is lowered by a factor of about two. In

TABLE VIII

Synergism of propylparaoxon and diazinon

Percentage mortality 24h. after the topical application of 1 μl of a solution in acetone

μg per fly of	S		C			Ka			Fa		Fc	
prop. paraox. / diazinon	0	0.1	0	0.1	0.2	0	0.1	0.2	0	0.1	0	0.1
0	—	4	—	5	13	—	5	4	—	5	—	0
0.007	—	25	—	—	—	—	—	—	—	33	—	—
0.015	0	78	—	—	—	—	—	5	—	82	—	—
0.03	43	93	—	—	—	—	5	75	5	90	—	—
0.05	93	100	—	40	35	—	30	90	35	100	—	5
0.1	100	—	—	40	75	3	85	100	93	—	0	30
0.2	—	—	—	70	80	93	100	—	100	—	0	40
0.5	—	—	15	83	80	100	—	—	100	—	60	90
1	—	—	35	100	100	—	—	—	—	—	95	—
2	—	—	70	100	—	—	—	—	—	—	—	—
4	—	—	100	—	—	—	—	—	—	—	—	—
8	—	—	95	—	—	—	—	—	—	—	—	—

strains C, Ka and Fa with constitution *aa* the factor is larger, indicating that *in vivo* inhibition of the breakdown enzyme is achieved. However, in strain C and Ka a significant degree of resistance is still present. Since resistance in strain Ka is known to depend exclusively on the breakdown enzyme, it must be concluded that under the experimental conditions this enzyme is not completely blocked. Propyl-paraoxon was also applied in the contact method. In this method resistance to diazinon brought about by gene *a* is much higher than in the topical application method (compare the resistance factors shown in Table II with those of Table VIII) and it was expected therefore that the synergistic effect would be larger in this method. When 30 μg of propyl-paraoxon per jar was combined with diazinon the LD_{50}'s for strains S, C and Ka were 1, 18 and 10 μg respectively. This means that the resistance factors of 50 and 40 were reduced to 18 and 10, and it can be concluded that also in the contact method no complete block of the breakdown enzyme is attained.

Experiments similar to those with propyl-paraoxon were carried out with n-propylparathion, n-butylparaoxon and n-butylparathion. The effect of propylparathion was slightly less, that of the butyl compounds much less than that of propylparaoxon. A very marked synergism in strains G and H was found with sublethal doses of paraoxon combined with malathion. A dose of 0.02 μg per fly was equivalent in its action to 0.1 μg of propyl-paraoxon. Apparently much less paraoxon than propyl-paraoxon needs to be applied to block the breakdown enzyme. These results indicate that properties affecting penetration and transport to the breakdown enzyme play a large part in determining the effectiveness of a compound as a synergist.

DISCUSSION

It is gradually becoming apparent that in the development of resistance in insects the role of major genes is more important than that of polygenes (MILANI, 1959; DAVIDSON & JACKSON, 1961). In this situation a genetic analysis can be an important tool in the search for physiological mechanisms that cause resistance. If a single gene is responsible for resistance in a certain strain, or if a strain can be experimentally made in which one gene for resistance is present with the exclusion of others, the study of the physiological basis is greatly facilitated. This is of special importance in an organism such as the housefly with its formidable capacity of genetic adaptation, where a complex pattern of resistance mechanisms can be present.

Isolation of single resistance genes and consequently of separate mechanisms is also important in the study of cross resistance patterns. Even if it is known that a certain type of resistance is mainly brought about by one gene, the possibility remains that genes of little importance for this resistance are responsible for a high degree of cross resistance to other compounds. In this connection it is interesting to note that organophosphate resistant strains often show a high level of cross resistance to chlorinated hydrocarbons whereas the reverse is not true (FORGASH & HANSENS, 1959; MARCH, 1960). The nature of the mechanism that contributes most to the organophosphate resistance, seems to preclude that it is of importance for the unrelated chlorinated compounds. Therefore, the most likely explanation seems to be that this cross resistance is brought about by genes with a relatively small effect on organophosphate resistance. A similar situation is probably found in the case of chlorthion cross resistance of strain G. Its malathion resistance is mainly due to the *a* allele. Since the breakdown enzyme formed by this allele is less effective toward chlorooxon than that of strain C, the fact that strain G has a higher resistance to chlorthion than strain C probably rests on other factors contributing only little to the malathion resistance.

The combination of genetical experiments with the study of breakdown of organophosphates *in vitro* as described here clearly shows that the main cause of OP-resistance is degradation of the organophosphate by some altered ali-esterase, although other mechanisms are present in some strains. This is corroborated by the strong synergistic action of organophosphates that block these breakdown enzymes. However, difficulties are encountered if one tries to bring this view into agreement with some data on the fate of these insecticides *in vivo*. Breakdown capacity that is measured *in vitro* is low as compared to the LD_{50} of the resistant strains, i.e. the altered ali-esterase can detoxify only part of a dose against which it apparently gives protection. It is known that a considerable breakdown by other mechanisms takes place both in susceptible and resistant strains. MARCH (1959) reported breakdown of malaoxon by sections of susceptible flies, KRUEGER & O'BRIEN (1959) found breakdown of malathion by susceptible flies, MENGLE & CASIDA (1960) breakdown of diazinon, malathion and methylparathion. KRUEGER & CASIDA (1961) showed paraoxon, DDVP and other OP-compounds to be degraded *in vitro*.

Mengle & Casida studied the fate of radiolabeled diazinon upon injection into flies of one susceptible and two diazinon resistant strains. One of the resistant strains (R-A) has the same origin as our strain K. According to our findings the ali-esterase-like enzyme for detoxication of diazoxon, present in this strain, is capable of degrading the dose applied by these authors (0.08 μg per fly) *in* *vitro* in about 2 hours. Nevertheless it seems to have contributed very little to the metabolism of the diazinon injected. In 0.5 and 1 hour 25 and 48% of the dose (approximately the LD_{50} for the susceptible strain) was degraded to water soluble breakdown products in the susceptible strain, and 28 and 48% in the R-A strain. In the same experiment 3.7 and 6.2% was present in the form of the oxygen analogue in the susceptible strain, and 2.5 and 5% in the resistant one.

Therefore we have to reconcile the fact that the ali-esterase-like breakdown enzymes are able to prevent the toxicant from reaching a fatal concentration at the site of action whereas most of the total elimination is brought about by other mechanisms present in both susceptible and resistant strains. This seeming discrepancy is more easily understood if the very high affinity of the breakdown enzymes and their low turnover number are considered. Low turnover could be the cause of the fact that the enzyme contributes only a little to the breakdown at high concentrations of organophosphate whereas its very high affinity may be of special importance in removing the toxicant at very low concentrations where other protective mechanisms may be ineffective. However, for a better understanding more should be known about localisation of sites of action, breakdown enzymes causing resistance and other elimination mechanisms.

It is noteworthy that the mechanisms for elimination of organophosphates which are present both in susceptible and in resistant strains are not active in homogenates, at least not with the low concentrations employed in this study. This has been of great help in the analysis of the resistance mechanism.

Propyl-paraoxon proves to be a potent synergist for malathion and diazinon resistant strains. The question arises whether synergists could be of practical value in control of OP-resistant flies. This will largely depend on the ease with which alternative resistance mechanisms will be selected. Examples of the existence of such factors have been found already and it is probable, therefore, that some resistance will develop to synergist combinations. Experiments aiming at the selection of such a strain are under way.

Acknowledgement

Thanks are due to Mrs. I. van der Linden-van Esch and Mrs. G. de Bie-Rike, Misses A. Talens and C. de Wilde, and Mr. H. Muller and Mr. A. Weyers for technical assistance.

ZUSAMMENFASSUNG

ABBAUENZYME ALS URSACHE DER PHOSPHORSÄURE ESTER-RESISTENZ BEI DER STUBENFLIEGE; EIGENSCHAFTEN, HEMMUNG UND DIE SYNERGISTISCHE WIRKUNG VON HEMMENDEN SUBSTANZEN

In phosphorsäureester-resistenten Stubenfliegen sind Abbaufermente vorhanden, die die Fähigkeit zur Hydrolyse von Sauerstoffderivaten derjenigen Verbindungen besitzen, gegen die Resistenz aufgetreten ist. Diese Enzyme entstehen an Stelle einer in anfälligen Fliegen vorkommenden Ali-Esterase. Das Auftreten der Ali-Esterase und der Abbaufermente unterliegt dem Einfluß verschiedener Allele eines Gens. Durch eine Kombination biochemischer, genetischer und toxikologischer Arbeitsmethoden wurden Bedeutung und Eigenschaften dieser Enzyme untersucht.

Die Hemmung der Ali-Esterase durch organische Phosphorverbindungen wird durch eine

irreversible Reaktion hervorgerufen, bei der eine Dialkylphosphorylierung des Enzyms stattfindet. Die modifizierten Ali-Esterasen oder Abbaufermente unterscheiden sich von der Ali-Esterase dadurch, daß der Reaktion mit den Produkten, gegen die Resistenz besteht, eine langsam fortschreitende Dephosphorylierung folgt. Diese Fähigkeit zur Hydrolyse bestimmter Verbindungen muß die Selektion der Allele hervorgerufen haben, die für die Bildung dieser Enzyme verantwortlich sind. Darüber hinaus wurden aber noch andere Veränderungen herbeigeführt: ein Verlust der Ali-Esterase-Aktivität, eine Änderung in der Affinität einer Anzahl Phosphorverbindungen gegenüber und eine Verminderung der Stabilität.

Vier verschiedene Abbaufermente sind in verschiedenen resistenten Stämmen vorhanden. Zwei von ihnen können Diäthylverbindungen mit einer Umsatzzahl von 0.05 und 0.25 pro Minute hydrolysieren. Das Enzym mit der höheren Umsatzzahl spaltet auch Dimethyl-Derivate; die Affinität für diese Verbindungen erscheint aber zur Ausbildung eines hohen Resistenzgrades zu niedrig. Zwei andere Fermente finden sich in Malathion-resistenten Stämmen, von denen eines wegen seiner wahrscheinlichen Instabilität in vitro nicht untersucht werden konnte. Das andere Enzym spaltet Malaoxon und einige weitere Methylverbindungen, wobei die Affinität Malaoxon gegenüber höher ist, was möglicherweise die Ursache der ziemlich hohen Spezifizität bildet.

Obwohl die Umsatzzahlen sehr niedrig sind und die Enzyme wohl nur einen geringen Teil des in vivo applizierten Giftes zu hydrolysieren vermögen, gestattet aber anscheinend ihre hohe Affinität für Phosphorverbindungen die für die Cholinesterasehemmung notwendige Inhibitormenge zu reduzieren und verursacht so die Resistenz.

Die Abbaufermente können bestimmte Typen von Phosphorsäureestern hydrolysieren; sie werden aber noch durch andere Verbindungen dieser Stoffklasse irreversibel gehemmt. Solche Präparate sind zur Blockierung der in vitro stattfindenden Hydrolyse befähigt und besitzen einen stark synergistischen Effekt, wenn sie mit den Thioverbindungen, gegen die Resistenz besteht, gleichzeitig appliziert werden. So werden die Abbaufermente der Malathion- und Parathion-resistenten Stämme durch Propylparaoxon irreversibel phosphoryliert. Sublethale Mengen dieser Substanz reduzieren die Resistenz beträchtlich.

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ERRATUM

MACLEOD, J. & DONNELLY, J. *Ent. exp. & appl.* 4 (1961): 108.

The second line of paragraph 3,
"good, but the total egg clusters obtained, over the ten days of test, were"
has been repeated from paragraph 1. The correct line should read
"density was made on the island and the adjacent mainland. 3,800 flies were"

BUCHBESPRECHUNG

The Ontogeny of Insects. Acta symposii de evolutione insectorum, Praha 1959. Publishing house of the Czechoslovak Academy of Sciences, Prague 1960. 406 Seiten mit zahlreichen Tabellen und Abbildungen.

Auf Einladung der Biologisch-Medizinischen Sektion der Tschechoslowakischen Akademie der Wissenschaften trafen sich Anfang September 1959 208, darunter fast 100 auswärtige Wissenschaftler aus 22 Ländern zu einer Vortragstagung über Probleme der Insektenentwicklung. Die Veranstalter, Mitglieder des Entomologischen Laboratoriums der Tschechoslowakischen Akademie der Wissenschaften, legen nun den Bericht über das Symposium vor, das mit Ausnahme einer eingeschobenen Panneldiskussion über entwicklungsphysiologische Probleme noch im traditionellen Stil abgehalten wurde, d.h. ohne Konzentration auf bestimmte Problemkreise und ohne entsprechende Selektion der Vorträge, deren Fülle das Programm so stark in Anspruch nahm, daß zu fruchtbaren Diskussionen selten Zeit blieb. Die größtenteils in extenso, zum Teil aber auch in stark gekürzter Form gedruckten 80 Vorträge aus den fünf Sektionen „Morphologie und Anatomie", „Physiologie", „Saisonale Periodizität (Diapause, Hibernation)", „Einfluß biotischer Faktoren" stellen deshalb ein sehr ungleichwertiges Mosaik von referatartigen Übersichten bis zu detaillierten Originalmitteilungen dar. Wie zur Zeit überall kommt auch hierbei das gesteigerte Interesse an biologischen Regulationen zum Ausdruck, sowohl an endogenen nervösen und inkretorischen Steuerungsprozessen mit ihren tiefgreifenden Einflüssen auf Wachstum, Differenzierung, Metamorphosen, Entwicklungsrhythmik und -ablauf, als auch an entsprechenden Wirkungen der unbelebten und belebten Umwelt auf diese Vorgänge und ihr kompliziertes Wechselspiel mit einander. Da die Synchronisation sowie die fremdsprachigen Übersetzungen während des Symposiums vielfach sehr unvollkommen waren, wird der vorzüglich ausgestattete Berichtband von allen Teilnehmern besonders dankbar begrüßt. Er bildet darüberhinaus ob seines Gehaltes an zahlreichen wertvollen Beiträgen ein unentbehrliches Quellenwerk für die interessierten Spezialisten, denn er bietet einen fast lückenlosen Überblick über das weitverzweigte Gebiet der Insektenontogenie.

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